Effect of UV-C treatment on sanitised and unsanitised ready-to-eat leafy green vegetables, produced in New Zealand

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ABSTRACT

Several outbreaks in ready-to-eat (RTE) salads have been reported with major concerns comprising of enteric pathogens such as Coliforms*,* *Listeria* (*L.*) *monocytogenes* and *Salmonella* spp. that have fast growth rates and low infectious doses. To improve the microbial safety of RTE salads, various methods have been used including non-thermal ultraviolet (UV) irradiation. Non-thermal UV irradiation causes fewer changes in the nutrition and sensory quality of food, compared to the conventional methods. This study investigated the effect of non-thermal UV-C dosage on microbiological levels of fresh commercial samples of sanitised (n=4) and unsanitised (n=4) spinach, kale, rocket and mesclun using 100 mJ/cm^2 dosage including controls. A commercial company in New Zealand supplied eight (n=8) freshly prepared commercial and packaged RTE salad samples. The sanitised samples had undergone normal preparation steps which included cutting, washing, and sanitation. The packaged samples (n = 8) were transported under chilled conditions (4°C) to Massey University Auckland Campus. Upon delivery, the samples were coded and treated by irradiation at 100 mJ/cm^2 (Radiant UV-21A0043, International Light Technologies, Inc. USA). The control and test (treated) samples were re-packaged in heat-sealed micro-perforated bags and stored for 12 days/4°C. Sample packages were retrieved on days 0, 4, 8 and 12 for analysis of total aerobic mesophilic counts (AMC), Coliforms, *L. monocytogenes, S. aureus* and *Salmonella* spp. using standard methods. The weight and colour (Minolta, Japan) of the samples were measured as well as evaluated by a focus sensory group for colour, texture, flavour, juiciness, firmness and aroma.

UV-C treatment (100 mJ/cm2) successfully reduced total AMC in all the sanitised and unsanitised salad samples (n=8) and no pathogens were detected during storage for 12 days/4°C. During storage, the AMC increased ($p<0.05$) for all the spinach samples including the UV-treated samples. However, the UV-treated samples had lower AMC than the control samples ($p<0.05$). Sanitisation of spinach samples had no effect ($p>0.05$) on the AMC. Kale samples recorded lower AMC for the irradiated samples ($p<0.05$) compared to control samples during storage. Higher AMC reductions were observed for the sanitised kale samples ($p<0.05$) than for the unsanitised samples. UV dosage, sanitisation and storage time had significant effects on AMC in mesclun samples ($p<0.05$) during storage. AMCs in UV-treated and sanitised mesclun salads were lower than in the control and unsanitised samples. The AMC of mesclun increased from day 0 to day 8 of storage, then decreased slightly on day 12. The AMC of rocket samples were similar to those of mesclun samples during storage. For rocket samples, AMC increased sharply from days 0 to 4, then slightly decreased from days 4 to 12. UV-treated rocket samples had lower AMC than the controls ($p<0.05$).

UV-C treatment of salads increased the lightness L* for all the samples except for rocket, decreased the yellowness b* for spinach and kale samples and did not affect the greenness a* of the salads. The lightness L* was lower ($p<0.05$) in treated spinach, kale and mesclun samples whereas, UV treatment and sanitization had no significant effect on the lightness L* of rocket samples during storage. UV treatment and sanitisation had no significant effect ($p>0.05$) on the greenness a* of spinach, kale, mesclun and rocket samples. The yellowness b* of spinach and kale was significantly affected by the dosage and the storage period ($p<0.05$) The b* value was low in treated spinach and kale samples compared to their respective controls. For mesclun and rocket, UV treatment had no significant effect ($p>0.05$) on the b* value. Sanitization had a significant effect on the rocket samples ($p<0.05$) as sanitised rocket had a lower b* value than the unsanitised rocket.

The effect of UV-C treatment on the weight loss (%) of salad samples was different for each salad. The weight loss (%) was higher in UV-treated spinach and kale samples than in the respective controls. Mesclun samples had the highest weight loss for control samples than UV-treated samples. For rocket salads, unsanitised controls (non-UV-treated) had higher weight loss than the unsanitised treated sample and the sanitised treated sample had slightly higher weight loss than the sanitised control sample.

Focus group sensory evaluation indicated that UV-treated salads had better taste than control samples, although the appearance, texture and colour were poorer than the non-UV-treated samples.

Overall, UV treatment reduced AMC in RTE salads without affecting the taste of the vegetables for 12 days/4°C. However, more work is required to maintain the overall appearance and texture of UV-treated salads.

[Keywords] sanitised and unsanitised salads, spinach, kale, mesclun, rocket, UV-C, microbial reduction, AMC (aerobic mesophilic count), weight loss, sensory analysis, colour analysis.

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Contents

List of Figures

List of Tables

Abbreviation

AMC: Aerobic Mesophilic Count

ANOVA: analysis of variance

APCs: aerobic plate counts

BPA: Baird-Parker agar

CDC: Centre for Disease Control

DNA: Deoxyribonucleic Acid

EFSA: European Food Safety Authority

EHEC: Enterohemorrhagic *E. coli*

FDA: Food and Drug Administration External

GMPs: Good Manufacturing Practices

HACCP: Hazard Analysis and Critical Control Points ()

HHHAB: High Humidity Hot Air Blanching ()

HHP: high hydrostatic pressure

HHP: high hydrostatic pressure

HPP: High-Pressure Processing

HUS: Hemolytic Uremic Syndrome

HVAD: high-voltage arc discharge

IRB: Infrared Blanching

MWB: Microwave Blanching

PAA: peroxyacetic acid

PEF: Pulsed electric field

PG: Polygalacturonase

PL: pulsed light

PME: pectin methylesterase

PPO: polyphenol oxidase

RF: Radio-frequency

RTE: Ready to eat

SK0: Sanitised kale control

SK100: Sanitised kale treated with 100 mJ/cm2

SM0: Sanitised mesclun control;

SM100: Sanitised mesclun treated with 100 mJ/cm2

SR0: Sanitised rocket control;

SR100: Sanitised rocket treated with 100 mJ/cm2

SS0: Sanitised spinach control

SS100: Sanitised spinach treated with 100 mJ/cm2

UK0: Unsanitised kale control;

UK100: Unsanitised kale treated with 100 mJ/cm2

UM0: Unsanitised mesclun control;

UM100: Unsanitised mesclun treated with 100 mJ/cm2

UR0: Unsanitised rocket control

UR100: Unsanitised rocket treated with 100 mJ/cm2

US: ultrasound

US100: Unsanitised spinach treated with 100 mJ/cm2

UV: Ultraviolet

VRBA: Violet Red Bile agar

XLD: Xylose-Lysine-Desoxycholate

1.0 INTRODUCTION

Interest in healthier lifestyles has led to changes in eating habits which in turn has increased the demand for ready-to-eat (RTE) salads and green leafy vegetables over the past 10 years (Soliva-Fortuny and Marti, 2003). As ready-to-eat fresh produce is consumed raw with minimal processing, they are associated with many food safety challenges (Little and Gillespie, 2008). Although minimally processed RTE vegetables are subjected to several processes and operations such as washing, peeling, chopping, sanitizing and packaging, they still pose food safety challenges with microbial contamination. Bacterial contamination of fresh leaves and salads has become a major issue and the contamination can occur while preparing, processing and handling the leaves (EFSA, 2008). Thus, there is a continuous search for better preservation techniques to improve the microbial safety of the products (FROeDER et al., 2007).

The consumption of minimally prepared fresh green leafy salads and vegetables has been associated with outbreaks of foodborne diseases (Taban and Halkman, 2011). For many foodborne diseases, fresh vegetables serve as potential vehicles which has led to some serious global outbreaks such as the contamination of spinach with *E. coli* and *Salmonella* in America (Campos, 2013). The occurrence of these pathogens in fresh produce has been often reported in many studies worldwide, highlighting the products as potential sources of human pathogens. Increased foodborne illnesses caused by pathogenic microorganisms, such as *Salmonella* spp., *Listeria monocytogenes* and pathogenic *Escherichia coli*, associated with the consumption of fresh and ready-to-eat produce have been widely reported (Berger et al., 2010; Callejón et al., 2015; de Oliveira Elias et al., 2015; Garner & Kathariou, 2016; Jung et al., 2014; Zhu et al., 2017).

In New Zealand, bagged lettuce mix produced by GSF Fresh New Zealand and sold under the brand names "LeaderBrand" and "Value" at various supermarkets throughout the country caused an outbreak of Yersinia pseudotuberculosis infections in 2018. This outbreak resulted in 20 people becoming sick (MPI, 2018). A total of 21 individuals in New Zealand were affected by an outbreak of Salmonella infections in 2019, which was linked to pre-packaged lettuce sold at Countdown supermarkets. The lettuce was imported from Australia and sold under the brand name "Wash N Toss" (MPI, 2019). The enterohemorrhagic *E. coli* (EHEC) outbreak in Germany in 2011 due to Spanish cucumbers, was declared the biggest outbreak ever in Europe, the second biggest worldwide, and the deadliest EHEC outbreak ever reported due to its size and virulence (World Health Organization, 2011).

Microbiological food safety has always been a focus of the food industry and public health agencies since foodborne pathogens cause many illnesses and deaths worldwide (Wadamori, Gooneratne & Hussain, 2017). In addition to the need for improvement in the overall quality of RTE vegetables and salads, faster rates of production and an increase in shelf life have led to the development of new technologies for the preservation of food (Olaimat and Holley, 2012).

Various developments in technologies focus on unit operations like sterilization, pasteurization, drying and cooking. The new food preservation technologies are a serious contender for the replacement of well-established traditional preservation methods (Morris et al., 2007). Food processors have a key goal to ensure the quality and safety of the technology before developing any new preservation techniques. For the microbial safety of food products, many industries employ thermal technologies which use heat to carry out operations like sterilization, pasteurization, evaporation and drying (Pereira and Vicente, 2010).

The conventional heat sterilization methods include the transfer of heat from a processing medium to the slowest heating zone of a product followed by cooling and it is one of the most common methods used for the preservation of food (Morales-de la Peña et al., 2019). Even though thermal sterilization is very effective in the inactivation of microorganisms, it can affect the quality of the product by changing its flavour, texture, colour and nutritional contents. The extent of these changes in the products depends on the type of product and the gradients in the temperature between food and process boundaries (Norton and Sun, 2008). Food processors are looking for a food preservation technology which guarantees and secures the integrity of the food by inactivating the pathogenic microorganisms without causing any changes in the nutritional and organoleptic qualities.

To reduce the impact of the treatment (e.g., thermal degradation) on the food and to enhance the physio-chemical properties, a variety of novel food preservation technologies have been developed over the past few years. The major goal of such innovations is to increase production and process efficacy with minimal or no variation in the colour or nutritional values of food, low consumption of energy and improvement in the shelf life of the product (Pereira & Vicente, 2010). The evolution of alternative novel technologies took place due to the loss of sensory and nutritional characteristics; high energy consumption, overcooking and consumers' need for minimally processed, healthy food. Some of the new non-thermal techniques used in the food industries for the inactivation of microorganisms are, chemical washing, high-pressure processing (HPP), electric and electromagnetic pulses, UV irradiation, ultra-sonication, pulsed light, microwave etc., and these are used for the microbial inactivation at ambient temperature (Buelvas-Caro et al., 2018., Priyadarshini et al., 2019).

UV technology is a promising non-thermal method for food processing and decontamination. Its ability to inactivate microorganisms without using heat makes it an attractive option for the food industry (Priyadarshini et al., 2019). UV is very efficient in the inactivation of pathogenic microorganisms and it causes minimum changes to the temperature and the organoleptic properties of the food product (Gabriel, 2012). However, it's important to consider factors such as surface characteristics and microbial resistance when implementing UV-C treatment.

To enhance the shelf life of RTE leafy green salads, combinations of chemical sanitizers like Tsunami 100 and physical treatments like UV-C irradiation is used as hurdle technology. Therefore, the study aimed to understand the effect of chemical and UV treatment individually and combined as a hurdle treatment to determine the synergistic antimicrobial effect. The salad samples were treated with different UV-C doses and the optimum dose was selected to study the shelf life of the samples during a storage period of 12 days at 4 °C. During the storage period, microbial, sensory and physiological analyses were used.

1.1 Aim

To determine the effect of UV-C treatment on sanitised and unsanitised ready-to-eat salad leaf products.

1.2 Objectives

The research was carried out in three phases as follows:

Phase I

- Microbial analysis of UV-C treated and untreated samples of sanitised and unsanitised salad leaves of spinach, kale, rocket and mesclun purchased from the local market.
- Selection of UV-C dosage ranging from 100-700 mJ/cm^2 with maximum microbial reduction of sanitised and unsanitised salad samples

Phase II

- Microbial analysis of sanitised and unsanitised salads from a local supplier in New Zealand using selected UV-C doses.

Phase III

- Analysis of microbial count, physiological properties and sensory attributes of standard UV-C treated and untreated samples of the leafy salads during refrigerated storage.

2.0 LITERATURE REVIEW

2.1 Ready-to-eat leafy salads

The consumption of ready-to-eat salad has grown as a good source of dietary fibre, carbohydrates, phytochemicals, minerals, and vitamins (Chun, Kim & Song, 2010). They are also a major source of antioxidants, anticarcinogenic phytochemicals and complex carbohydrates, which are important for human health and well-being. Consumers are increasingly aware of the relationship between diet and health, and the trends in consumer purchasing patterns indicate a growing interest in fruits, leafy vegetables and salads (Charles & Arul, 2007). Leafy vegetables are important components of the diet, and their regular consumption in adequate amounts can prevent the onset of cardiovascular diseases and some types of cancers (Taban and Halkman, 2011).

As the leafy vegetables can be directly consumed eliminating the cooking process, they are considered ready-to-eat (RTE) foods and are growing commercially (Chaves et al., 2016). For health benefits, consumers have shown more interest towards chemical or preservative-free, convenient, fresh and healthy RTE vegetables over the past 20 years. RTE vegetables can be easily grown, and their cultivation can offer many opportunities for employment and address poverty. They have a short shelf life due to high water content and metabolic activity post-harvest, which makes them extremely perishable, especially in tropical temperatures (Gogo et al., 2017).

The safety and shelf life are the major factors that need to be increased with the increasing demand for fresh ready-to-eat vegetables. The natural variability that is observed in leafy salads mainly limits their shelf life and this is the biggest issue faced by the commercial industries (Allende et al., 2006). There are many chances of contamination by pathogens as fresh-cut vegetables are processed by removal of their natural barriers and hence, they aid the transmission of foodborne pathogens (Martínez-Hernández et al., 2015). Apart from the high demand for healthy and high-quality vegetables and fruits, the consumers also demand food free from chemical residues, microbial toxins and pathogens. Vegetables and fruits are highly vulnerable to transpiration, senescence, and infections due to fungi. For the extension of the shelf life, essential phytochemical retention and prevention from contamination and infection of vegetables and fruits, many effective and safer preservation methods are being employed by the horticulture industries (Charles & Arul, 2007).

2.2 Outbreaks due to salad consumption

Microbial hazards are causing many foodborne diseases which is becoming a serious public health issue. In the past two decades, the total number of cases reported due to microbial outbreaks has increased throughout the world. This may be caused because of various aspects such as global trade and an increase in travel, along with other factors like changes in food technology, agronomic processes, feeding practices, animal husbandry and the ageing population. Moreover, the increase in food-borne diseases has to be partially due to the changes in the lifestyle and demands of the consumers along with raising susceptible populations (Cabedo et al., 2008).

The cases of gastroenteritis outbreaks have increased throughout the world due to the intake of vegetable salads containing food-borne pathogens like diarrheagenic *E. coli* (Castro-Rosas et al., 2012). The food industries, consumers and governments are highly concerned with the increased number of outbreaks of food-borne diseases over the last 15 years due to the consumption of fresh vegetables. The advances in microbiological methods and surveillance programs have helped to find the association between food-borne pathogens and vegetables and it reflects the need to increase the microbial safety of fresh vegetable products. Out of various types of fresh products that are available in the market, RTE (ready-to-eat) vegetables have a major role in numerous outbreaks. Pathogenic *E. coli* and *Salmonella* were identified as the major food-borne pathogenic bacteria by epidemiological investigations that caused outbreaks (Sant'Ana, Franco & Schaffner, 2014).

There is an increase in the number of foodborne diseases caused due to consumption of RTE salads worldwide. According to studies, *Listeria monocytogenes* and *Escherichia coli* O157:H7 are the major contaminants that are found in RTE salads. Due to being highly virulent, the presence of *E. coli* in RTE salads is considered a serious issue (Chun, Kim & Song, 2010).

According to the data from the European Food Safety Authority, the proportion of reported listeriosis cases increased from 25.0% to 43.1% from 2012 to 2016 in Europe. In 2016, there were 1524 confirmed cases of listeriosis with 247 deaths. Since 2008, the annual death record due to listeriosis increased steadily with an average of 187 (EFS, 2017).

The fresh, raw produce can be contaminated by pathogenic and spoilage microorganisms like *Listeria monocytogenes, Escherichia coli, Staphylococcus aureus, Bacillus cereus* and *Salmonella enterica* during pre-harvest or post-harvest factors (Bhullar et al., 2018).

In the year 2013, a total number of 818 outbreaks of foodborne diseases were reported in the United States, which caused infection in 13,360 people and 16 deaths. In the outbreak, 34% of the cases that were reported were due to *Salmonella* which was the second major cause of the outbreak (Chaves et al., 2016). According to the data by Taban and Halkman (2011), ready-to-eat leafy salads, especially spinach and lettuce had increased association with the outbreaks due to foodborne diseases. These outbreaks may be because of contamination of microorganisms due to untreated water that may contain pathogenic microorganisms. Moreover, the salads can be contaminated by coming in contact with pathogens during harvesting, processing, packaging or handling (Chaves et al., 2016).

The U.S. Food and Drug Administration External (FDA) reported an outbreak of Shiga toxin-producing *E. coli* O157:H7 (STEC O157:H7) infections on November 2017, in multiple states of Canada and US due to the consumption of leafy green vegetables. Fifteen states reported 25 cases of infection out of which, 9 people were hospitalized and one death was reported (Centers for Disease Control and Prevention, 2018). On August 2020, 19 states reported an outbreak of *E. coli* O157:H7. A total number of 40 people were infected due to the consumption of leafy green salads. Twenty people were hospitalized, and four developed hemolytic uremic syndrome (HUS) with no deaths reported (Centers for Disease Control and Prevention, 2020). Packaged green leafy salads caused an outbreak of listeriosis in the US and Canada during 2015-2016. Nineteen cases were reported in the US and fourteen cases were reported in Canada by The Public Health Agency (Self et al., 2019).

A multistate outbreak of *E. coli* O157:H7 infections caused due to pre-packaged baby spinach was investigated by the CDC (Centre for Disease Control) and the U.S. Food and Drug Administration (FDA). Illnesses started on dates ranging from October 13, 2021, to November 8, 2021. A total of 15 people infected with the outbreak strain of *E. coli* O157:H7 were reported from 10 states, out of which four were hospitalized and three developed a type of kidney failure called hemolytic uremic syndrome (HUS). No deaths were reported (Centre for Disease Control and Prevention, 2022).

2.3 Sources of Contamination and Prevention

The pathogenic microorganisms can potentially contaminate fresh leafy vegetables and multiply during the preharvest and postharvest procedures like, processing, packaging, transportation and distribution.

When a vegetable or fruit is contaminated by human pathogens during growth and harvest, then it's known as primary contamination., and when it is contaminated during the process of cleaning, soaking, cutting, processing, packaging and transporting, it's called secondary contamination (Harris et al., 2003). The soil and the water used for irrigation or washing can naturally contaminate fruits and vegetables (Kiran, Anu & Dilip, 2001).

Ready-to-eat salads can be consumed raw and hence, do not require cooking, which in turn eliminates the microbiological step of killing. The growth and survival of pathogenic microbes may be enhanced due to further processing, transportation and storage conditions of vegetables and fruits (Kiran, Anu & Dilip 2001; Portman et al.,2002).

Various types of deterioration or contamination can be induced by the vegetable's chemical composition and physical structure. The microflora of the fruit or vegetable is also influenced by seasonality (Downes and Ito, 2001). Fresh produce can be potentially contaminated by anything it encounters. Major sources for contamination of fresh vegetables and fruits with foodborne microorganisms are animal and human faeces. The potential for contamination can be determined by the source and quality of water that is used for fresh crops and plants (Gorny, 2005).

Pathogenic microbes can live on plants for over 10-12 years. (Ivanek et al., 2006). If *Listeria monocytogenes* is present in the sludge, it can be viable for a long period in the soil (Kiran, Anu & Dilip 2001). *Listeria monocytogenes*, *Clostridium* spp and *Bacillus cereus* are some of the gram-positive bacteria which may be harboured by leafy vegetables when they come into contact with the soil (Downes and Ito, 2001).

The biofilm formed by microorganisms on the vegetable surface and the vegetable's serous cuticle protects the microorganisms and helps them to survive during washing and sanitization procedures. Vegetables that are chopped or have cuts are more prone to microbiological and chemical deterioration as the cuts cause damage to the cells, which in turn releases exudates rich in sugars, vitamins, minerals and other compounds. Pathogens that survive the processing step can grow using these conditions and nutrients (FROeDER et al., 2007).

The farmers may use fertilizers or manures which could have faecal contamination and hence t can serve as a source for contamination of vegetables. To avoid such contaminations, manure treatment is important. In vegetables, the probable presence of pathogenic microorganisms can be determined by the factors like the quality of irrigation water and treated manure. As compared to the direct infection on the leaves, contamination caused by *Listeria monocytogenes* and *Salmonella* spp through roots is significantly higher. The growth and multiplication of pathogens can also be favoured by natural sources of water like rain. During the process of handling, storage and distribution, bacteria can adhere to the surface of the vegetable succeeding in internalization and proliferation of pathogenic microorganisms if the vegetable is in contact with contaminated irrigation water. Foodborne pathogenic contamination of vegetables can also be affected by season and climate change along with factors like longitude and latitude which constitute geographical differences (Matthews et al., 2017; Liu et al., 2013).

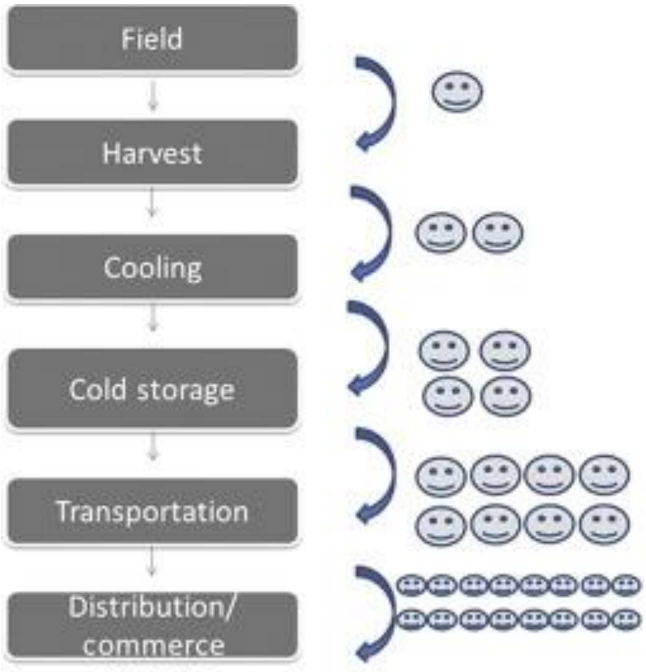


Figure 2.1 Schematic presentation of bacterial multiplication throughout the production chain.
Source: Chaves et al., 2016

Some major sources of microbial contamination according to Portman et al., (2002), which causes food safety risks are as follows:

- Soil and sludge
- Fertilizers and manure
- Pesticides, chemicals and insecticides
- Water

- Birds and animals
- Vermin and pets
- Equipment and machines
- Personnel
- Packaging materials
- Discarded fruit and plant debris

From the harvest of vegetables to its distribution, the fundamentals to prevent contamination are primarily sanitary and hygienic conditions in the cold chain management and processing industry. The contamination on the farms can be minimized by emphasizing hazard analysis and critical control points (HACCP) and good manufacturing practices (GMPs), which are generally followed in the food processing industries (Ivanek et al., 2006). According to Chaves et al., (2016), the precautionary measures that need to be applied for ready-to-eat products during all stages of production are:

1. Following good agricultural practices during primary production can reduce the risk of contamination from animal waste, pesticides, or irrigation water.
2. Cleansing and disinfecting the processing instruments and equipment can prevent cross-contamination during processing. Regular cleaning and disinfection can help prevent the buildup of bacteria, viruses, or other pathogens on surfaces and equipment.
3. Thermal and non-thermal processes should be employed for the reduction of viable cells and pathogen levels.
4. The cleaning and disinfection process should be validated by using microbiological testing as it ensures that the cleaning and disinfection processes are effective in reducing pathogen levels.
5. Educating stakeholders is an important point because food safety is a shared responsibility among all parties involved in the food production and distribution chain. Educating all stakeholders, including farmers, processors, distributors, and consumers, about proper food safety practices can help prevent contamination and reduce the risk of foodborne illness.

To minimize food safety hazards caused by microorganisms and reduce risks to the environment and food supply, growers and handlers of fresh produce should follow good agricultural practices (Gorny, 2005). By implementing control measures during both pre-harvest and post-harvest stages, a strategy for preventing microbial contamination during fruit and vegetable production can be developed. Proper management of processes that involve the use of manure and municipal biowastes can minimize the possibility of microbial contamination of fresh food products. To further minimize the risk of foodborne pathogen contamination, hygiene and sanitation should be practised during harvesting, sorting, packing, and transportation (Gorny, 2005).

The growers and handlers of fresh vegetables and fruits have numerous documents for guidance which are developed to reduce microbiological hazards. Based on the fundamentals of good hygienic practices, good agricultural practices, good handling practices and the HACCP system (hazard analysis critical control point), control strategies are developed and incorporated into the documents (Chaves et al., 2016). For an effective food safety program, all applicable federal, state and local laws and regulations and standard agriculture practices should be followed. Various levels of production like farms, packaging departments, distribution facilities and transport centres should be accountable. For the proper functioning of the program and to track back the products to the producer via distribution facilities, effective monitoring and qualified employees are essential (Gorny, 2005).

2.4 Food preservation techniques

Foods are naturally perishable. The quality of the food is affected by the reactions caused due to microbiological, physical, chemical and enzymatic changes. These deteriorative reactions can be prevented by employing food preservation techniques which will help to extend the shelf life of the food and keep it safe for consumption (Raso and Barbosa-Cánovas, 2003). Spoilage can make the fresh produce lose its edibility, colour, texture and nutritional values

One of the major concerns of food industries is the safety of food. Food safety can be maintained by scrutinizing the materials that enter the food chain, suppressing the growth of microorganisms using temperature control, and by reducing the microbial load to prevent pre and post-treatment contamination. The preservation technology used should be efficient in inhibiting pathogens and chemical deterioration but, at the same time, it should not compromise the organoleptic and physical features (Critzer et al., 2007; Prokopov and Tanchev, 2007; Lado and Yousef, 2002). Based on consumer satisfaction in terms of sensory, nutrition, safety and the need for economic preservation, many new preservation technologies have been developed (Prokopov and Tanchev, 2007). According to Raso and Barbosa-Cánovas, (2003), an ideal food preservation technology should inhibit the growth of spoilage and pathogenic microorganisms and extend the shelf life of the food. It should be easy to use and economical. It should not cause any changes to the nutritional and organoleptic properties of food. The treatment should not leave any residue and it should have no objections from the legislators and consumers.

Thermal treatment is a traditional method that is widely used by the food industries to kill and inactivate microorganisms (Manas and Pagán, 2005). There are various types of thermal treatments which include, sterilization, pasteurization, evaporation and drying. In thermal technology, heat is generated outside the product that needs to be sterilized by using an electric resistive heater or combusting fuel. The heat is transferred to the product by conduction or convection (Vicente and Castro, 2007). Novel thermal technologies include microwave heating, dielectric heating and ohmic heating which uses radio and microwaves to generate heat. While these technologies are good alternatives to the traditional thermal process due to their ability to generate heat directly inside the product, they do come with certain drawbacks that must be taken into consideration (Pereira and Vicente, 2010). The microstructure of fresh produce can be destroyed by these methods as they cause loss of moisture.

Microwave blanching (MWB) is a preferable method for small or thin vegetables and fruits. However, one disadvantage of MWB is its uneven heating, which can result in unwanted colour changes, localized moisture dispersion, and nutrient leakage. The recently developed heat pre-treatment processes, such as MWB and Infrared blanching (IRB), have limitations in terms of their ability to penetrate deeply and the longer duration required for High humidity hot air blanching (HHHAB). These limitations can cause tissue degradation and negatively affect the bioactive substances present in the food (Boateng, 2022). Furthermore, novel technologies like Radio-frequency (RF) heating may struggle to evenly distribute heat within solid or semi-solid foods due to their low thermal diffusivities. Prolonged exposure to high temperatures can also lead to a decline in the quality of the food structure (Jiao et al., 2018). Thermal techniques have the potential to induce heat-related reactions that impact the flavour, aromatic compounds, and overall sensory quality of food. These reactions include the breakdown of lipids through thermal degradation, dephosphorylation of proteins, hydrolysis of peptide bonds, Maillard reactions, and the interaction between lipid oxidation and Maillard reaction products (Kamani et al., 2019).

Although thermal technologies are effective in inhibiting the growth of pathogenic microorganisms, they have drawbacks such as the loss of functional properties and negative effects on the wholesomeness, texture, colour, flavour, and nutritional qualities (such as vitamins and proteins) of food. These methods are not practically applicable to fresh produce. Thermal pasteurization technology affects factors such as non-enzymatic browning, protein denaturation, enzyme inactivation, and lipid oxidation. Moreover, certain heat-sensitive polyphenols that contribute to the food's quality can be destroyed during thermal treatments (Montenegro et al., 2002; Morris, Brody, and Wicker, 2007; Zhang et al., 2019). Conventional methods of food preservation, including pasteurization, chilling, freezing, drying, and chemical preservation, are widely used globally (Amit et al., 2017).

Chemicals and fumigants are used in produce industries to prevent pathogenic microbes in packing and handling industries (Montenegro et al. 2002). Chemicals like ozone, organic acids, peroxyacid, hypochlorites, ethylene oxide, chlorine dioxide, anhydrous ammonia and acidified sodium chlorite are used by food industries to prevent spoilage caused by microorganisms and to eradicate pathogens. The chemicals used should not compromise the legality, integrity, and safety of the fresh produce. Factors like lethality, antimicrobial spectrum and food’s chemical composition decide the concentration of the chemical used (Gurnari, 2015).

Innovations and the evolution of current technologies are aided the progress and advancement in science. Alternative methods of thermal treatment have been developed for the reduction and inhibition of microbial population without compromising the quality and to overcome the limitations of thermal technology. Novel non-thermal technologies are gaining much importance due to global interest in minimally processed, fresh and natural products (Amit et al., 2017; Pereira and Vicente, 2010; Critzer et al., 2007; Manas and Pagán, 2005).

Pulsed electric field (PEF), pulsed light treatment (PL), high-pressure processing (HPP), high hydrostatic pressure (HHP), ultrasound under pressure, ionizing irradiations and hurdle technology are some of the examples of novel non-thermal technologies that have gained much importance. These technologies help in the inactivation of pathogens at ambient temperature which prevents the components of food from thermal degradation, protecting the nutritional and sensory properties of the fresh produce (Pereira and Vicente, 2010; Manas and Pagán, 2005).

The spoilage or deterioration caused due to microbiological, chemical and enzymatic changes can be inhibited by chemical modification, gas removal, structure modification, surface coating, antioxidants, chemical preservatives, fermentation, acidification, increase CO_2, decrease O_2, reduce water activity, freezing and low temperatures. The microorganisms can be inactivated by chemical preservation, pasteurization, high pressure, PEF, radiation and sterilization. Recontamination of food products can be prevented by sanitary treatment, cleaning and packaging (Prokopov and Tanchev, 2007).

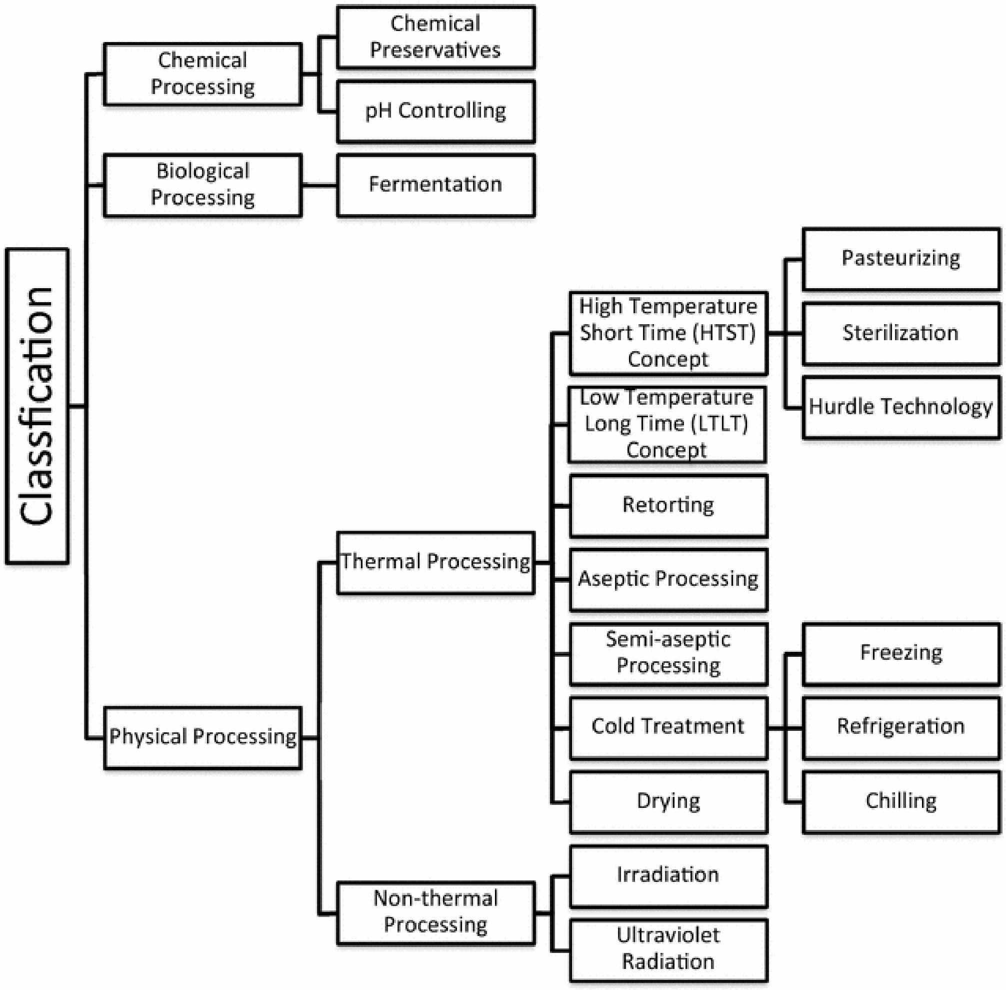


Figure 2.2 Different methods of preservation techniques.
Source: Amit et al., (2017)

2.5 Non-thermal technology of preservation

Over the past 25 years, there has been exponential growth in the need for diverse and convenient food products along with the demand for enhanced quality, extended shelf life and faster rates of production. All these factors along with the shortcomings of traditional preservation technology have led to the enhancement of current technology and the development of new preservation techniques (Pereira and Vicente, 2010).

Technologies that don't cause the inactivation of microorganisms and enzymes using heat as means are known as non-thermal technologies (Lopes et al., 2018). The non-thermal method of preservation extends the shelf life of the food by killing or inactivating microorganisms at sublethal temperatures, therefore, causes the least effect on the sensory, functional and nutritional attributes of the food, due to which many manufacturers, food researchers and consumers are interested in it (Morris, Brody and Wicker, 2007; Manas & Pagán, 2005). Temperatures used by non-thermal technologies are lower than that used for thermal pasteurization, hence at the time of processing, minimum or no changes occur to the flavour, essential vitamins and nutrients of the food (Barbosa-Cánovas et al., 2005).

High food safety standards are maintained by non-thermal technologies and they also meet the consumer's demand for minimally processed food with minimum effect on the food's nutritional value, taste, aroma and colour (Lopes et al., 2018). In non-thermal technologies, the shelf life is longer, treatment time is shorter, energy efficiency and safety levels are higher and better-quality attributes are preserved in comparison to conventional preservation methods like drying, evaporation and pasteurization (Zhang et al., 2019; Lopes et al., 2018; Morris, Brody and Wicker, 2007).

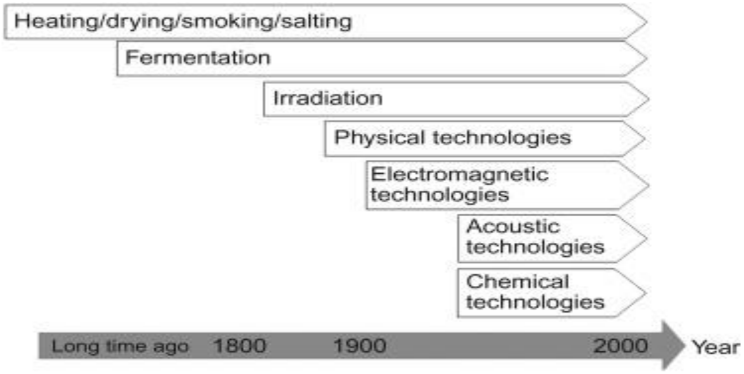


Figure 2.3 Various food preservation techniques over the years
Source: Barba et al., (2018).

Non-thermal processing of food can be done through various methods like irradiation which includes X-rays, gamma rays and ultraviolet (UV) light sources; electrical treatments like high-voltage arc discharge (HVAD), pulsed electric field (PEF) and pulsed light method (PL); high-pressure processing (HPP) or high hydrostatic pressure (HHP), ultra and micro filtration technique, ultrasound (US), chemical treatment, use of antimicrobials and bacteriocins; and hurdle technology (Choudhary and Bandla, 2012; Pereira and Vicente, 2009; Critzer et al. 2007). These non-thermal technologies can be used on their own or by combining with other technology for a variety of solid and liquid foods to enhance the inactivation of microorganisms, improve the quality and reduce the time for processing (Barbosa-Cánovas et al., 2005).

Pulsed electric field - It inactivates the pathogenic microorganism through a method called electroporation in which the cell membrane of the microorganism is disrupted due to which the functionality of the cell membrane is lost. This leads to an irreversible change and microbial inactivation (Sharma et al., 2014; Castro et al., 1993).

High voltage arc discharge - In this technique, the cell membrane of the microorganism is ruptured mechanically due to which its intracellular compounds are extracted. High voltage arc discharge stimulates free radical formation like oxygen from the food. Some intracellular components are inactivated due to the free radicals which act as toxins and stop the cell metabolism. The changes caused to the microbial cell are irreversible and hence cause permanent damage to the cell (Stoica et al., 2013).

High-pressure processing – In this technique, the permeabilization of the cell membrane is the cause of the death of microbial cells. Irreversible changes occur to the morphology of the cell when high pressure is applied and hence causing cell death (Rendueles et al., 2011; Rastogi et al., 2007).

Cold plasma – This method can help in the inactivation of many pathogenic microorganisms, molds, yeasts, bacteria, biofilms and spores. The DNA, protein and lipid bilayer of the microbial cell is damaged by this method and in turn, it kills the microorganisms (Lacombe et al., 2015; Tani et al., 2012).

Ultrasound – This technology causes the disruption of the microbial cell by cell membrane thinning and damaging of the cell wall. Free radical production and localised heating also cause the inhibition of microbial growth (Chemat and Khan, 2011).

Irradiation – The cellular nuclei of the microbial cell is attacked by the radiation which causes changes in the cytoplasmic membrane of the cell and the chromosomal DNA. DNA fragmentation is the main reason for the death of microbial cells (Feliciano et al., 2014; Shea and Committee, 2000).

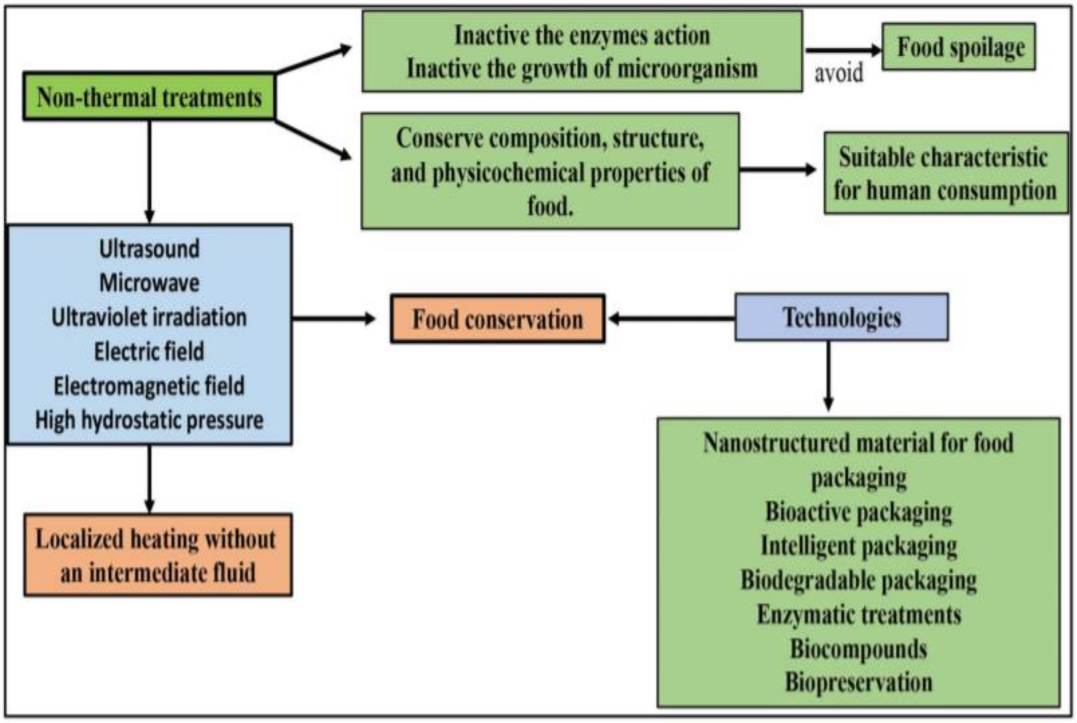


Figure 2.4 Different types of non-thermal food processing technologies
Source: Buelvas-Caro, Assia-Ortiz and Polo-Corrales, (2018).

2.6 Chemical Sanitation

Product safety is ensured in the fresh produce processing through unit operations such as disinfection and cleaning. Chemical and physical treatments are used during the process of washing for the elimination and reduction of pathogens from food (Moscetti et al., 2013). Product quality and shelf life should not be affected by such treatments. Cleaning, washing and sanitisation are essential for microbial quality and shelf life of food. Spoilage or pathogenic microbes can be eliminated from the food surface using sanitizers and disinfectants (Joshi et al., 2013).

Fresh produce manufacturing industries widely use chlorination as a chemical disinfectant for the reduction of contamination by microorganisms in fresh food products (Beuchat, Adler & Lang, 2004;

Villanueva et al., 2004). Chlorine is commonly used as sodium hypochloride (NaOCl) to treat fruits and vegetables as it is economical and has efficient antimicrobial activity. Perishable products contain organic components which react with chlorine and form by-products that can be potentially carcinogenic and toxic, this has raised a lot of concerns among the consumers and food industries (Villanueva et al., 2004). Toxic halogenated by-products are formed when chlorine reacts with the food's organic compound and trihalomethanes and disinfecting chlorine are formed by the reaction between hypochlorous acid and organic substance (Joshi et al., 2013; Ivancev-Tumbas et al., 1999). Thus, an alternative effective and safe disinfectant is required to replace chlorine to protect the quality of the food.

Fresh produce can be effectively sanitised using a peracetic acid-based sanitizer known as Tsunami 100. It acts as an efficient bactericide and fungicide. Peroxyacetic acid also known as peroxyacetic acid (PAA) and hydrogen peroxide are the active compounds that makeup Tsunami 100 (Allende et al., 2008). The US Food and Drug Administration approved the use of peracetic acid-based sanitizer for fresh produce (Ruiz-Cruz et al., 2007). The use of PPA based additive, Tsunami 100 was approved by the Environmental Protection Agency and Spanish regulations, for the treatment of drinking water, vegetables and fruits (Alvaro et al., 2009; EPA, 1998).

PAA inhibits the bacterial cell by attacking majorly on the lipoproteins present in the outer and inner cell membranes (Alvaro et al., 2009). *L. monocytogenes*, *Salmonella enterica* and *E. coli* can be reduced by 99.9% in the fresh-cut produce and the processing water system using Tsunami 100. Apart from the inhibition of pathogens that cause decay and spoilage, it even controls the effect of spoilage triggered due to non-pathogenic microbes and hence, helps in the extension of the shelf life of fresh vegetables and fruits (Botondi, Moscetti, & Massantini, 2016). According to Artés et al. (2007) and Rodgers et al. (2004), Tsunami 100 is ideal for the treatment of horticultural products as it has tolerance to various factors like pH, hardness of water, temperature, activity biocide and soil contamination.

As per the studies conducted by Neo et al. (2013), Tsunami 100 had a slightly better effect on the reduction of *L. monocytogenes*, *E. coli* O157:H7, *Salmonella* spp. and native microflora than chlorine. In comparison to chlorine, PAA does not have weak activity when organic compounds are present, and they do not form any carcinogenic or toxic by-products by reacting with the protein or organic matter of the food (Vandekinderen et al., 2009).

The detrimental impact of PAA is comparatively less on consumer health and the environment than chlorine. Once the chemical disintegration and disinfestation are completed, PAA breaks into acetic acid and oxygen, which further breaks into water and carbonic anhydride. The decomposition of PAA causes the formation of biodegradable acetic acid and it finally enters as atomic oxygen in the medium therefore, these by-products are environment friendly (Alvaro et al., 2009). Hence, it can be concluded that Tsunami 100 is a potential replacement for widely used chlorine, as it is more efficient and does not compromise with the quality of fresh produce.

2.7 Hurdle Technology

Various factors (hurdles) are combined during the processing of many traditional and novel food products to prevent microorganisms from overcoming these hurdles and in turn increase the microbial safety and stability of the food. This is known as the hurdle effect and it plays a vital role in food preservation (Leistner,1995). As different technologies are combined and used in hurdle technology, it displays a synergistic effect to inhibit the microbes (Rahman et al., 2016).

For food preservation, hurdle technology plays a fundamental role as the hurdles incorporated can prevent spoilage by microorganisms and food poisoning caused due to it, maintain the quality of the food produced and extend its shelf life (Leistner, 1995). Besides the sensory quality and microbial safety, the economic and nutritional properties of the food can be improved by combining various hurdles (Leistner & Gorris, 1994). Preservation technologies that are novel and existing are combined to create a sequence of preservation factors (hurdles) that hinders the microorganism from increasing. Preservatives, pH, water activity (a_w), temperature and redox potential can serve as hurdles. The number of hurdles is directly proportional to the efforts required by the microorganism to overcome them (Leistner & Gorris, 1995). Hurdle technology provides a preservation effect that is mild, and multi targeted but reliable.

The nutritional values, texture and sensory attributes of the food can be adversely affected by some technologies. This could be controlled by employing hurdle technology. Sensory and organoleptic properties can be improved by using this combination strategy. The increase in product quality, productivity and reliability, and the decrease in energy consumption and emission can be achieved by using novel food preservation technologies like the pulsed electric field, electrolysed water, high-pressure processing, irradiation technologies and ohmic heating. The adverse effect of treatment on fresh produce, the cost of sterilization and the quantity of sanitizer can be reduced, and the shelf life of the produce can be extended by combining two or more preservation technologies (hurdles) (Khan et al., 2017). One suitable hurdle that can be combined with chemical treatment is UV treatment, which can be used to improve the quality and safety of food.

2.8 UV Treatment

Ultraviolet light (UV) irradiation is a traditional non-thermal treatment, which is used for the decontamination and disinfection of water, air and surfaces. Disinfection of food products using UV technology is very effective as it kills microorganisms without using heat (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000).

In the food processing industries, UV treatment can be used as a promising alternative to traditional thermal treatment. UV treatment is used for the extension of the shelf life of fresh or raw products, treatment of surface of the food contact, meat treatment post lethality and juice pasteurization. UV treatment has considerably great potential in the food industry due to the growing concerns and negative reactions of consumers towards chemical additives in food. Consumers have a positive response towards UV treatment as it's a physical method of preservation (Shah et al., 2016; Koutchma, 2008). UV irradiation is declared safe for the treatment of food products by the US Department of Agriculture (USDA) and the U.S. Food and Drug Administration (FDA). For the treatment of fresh juices, UV treatment was approved as an alternative to thermal pasteurization by the FDA in 2000 (US FDA, 2000).

The UV treatment has gained a lot of interest from the food industries and researchers as an emerging non-thermal technology for the sanitization of fresh produce as it can effectively inactivate a broad range of pathogenic and spoilage-causing microorganisms and has a minimum effect on the food's sensory and nutritional qualities. The UV-C technology is user-friendly, economical, energy efficient and easy to maintain (Keyser et al., 2008).

As compared to other thermal and non-thermal technologies for food disinfection, UV irradiation consumes less energy and does not produce any residue or cause any toxic effect (Gayán, Condón &

Álvarez, 2014). In comparison to ionizing radiation like gamma, UV irradiation does not produce any radioactivity residual and hence, it is a non-ionizing intervention technology (Park, Kang & Kang, 2018). The processing of food using UV irradiation has turned out to be great innovative technology with a high potential for commercialize_(Morales-de-la et al., 2019).

Figure 2.5 Characteristics of UV-C treatment
Source: Delorme et al., (2020)

LEDs (light emitting diodes), pulsed light, low-pressure mercury lamps (LPM) and medium-pressure mercury lamps (MPM) are some sources of UV light that are available commercially (Koutchma, 2009). Light in the germicidal range is emitted by these lamps which are used for medical purposes and disinfection of food. Continuous UV radiation is produced by mercury (Hg) lamps and pulsed UV light is produced by flash lamps filled with xenon (Xe) (Barba et al., 2018). UV radiation is a form of invisible light. In the electromagnetic spectrum, UV light contains a wide non-ionising region between visible light (400 nm) and X-rays (200 nm). The UV light spectrum contains the following regions:

- UV-A: long waved ranging from 315 to 400 nm.
- UV-B: medium waved ranging from 280 to 315 nm.
- UV-C: short wave ranging from 200 to 280 nm.

- Vacuum UV: ranging from 100 to 200 nm (Shin et al., 2016; Bintsis et al., 2000).

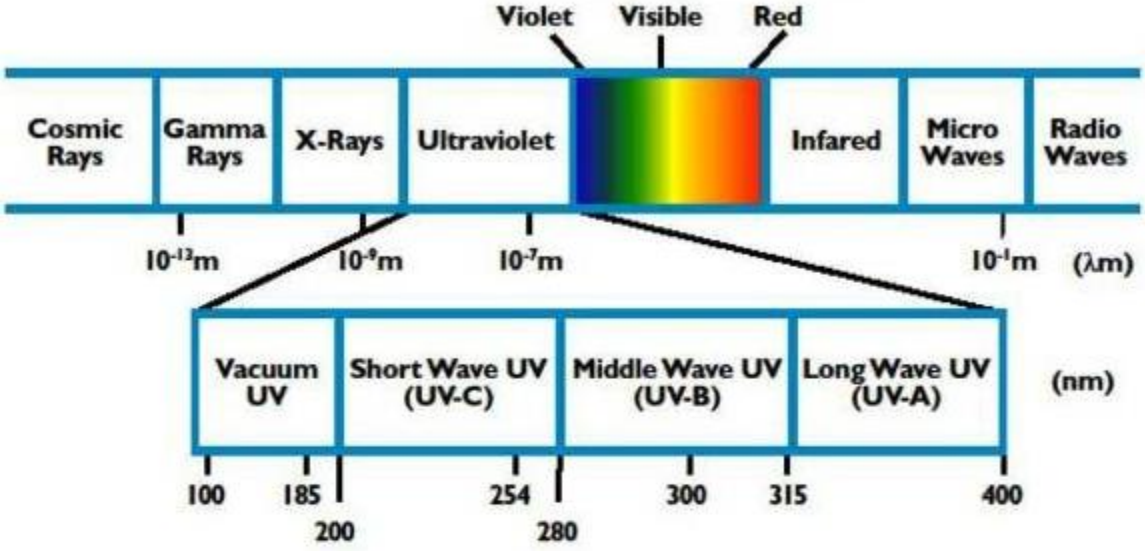


Figure 2.6 UV in the Electromagnetic spectrum
Source: https://materion.com/

The UV-C region of the UV light displays the germicidal effect. UV-C exhibits high lethality towards various microbes like fungi, bacteria, algae, protozoa and viruses therefore, UV-C is a germicide. At approximately 254 to 264 nm range, maximum inactivation occurs which forms a bell-shaped curve of inactivation efficiency (Shin et al., 2016).

When the food produce is treated with UV-C radiation, the radiation is absorbed by the DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) of the microbial cell. The UV-C radiation causes bactericidal effect by the formation of photoproducts that interrupts the process of transcription and translation of DNA which leads to cell death of the microorganism (Cheigh et al., 2012). The primary mechanism that occurs after the absorption of UV-C is the formation of pyrimidine dimers which, inhibits the replication of microbial cell and cause inactivation of the microorganisms and hence, prevent the infection (Harm, 1980).

The UV-C radiation primarily targets the genetic material of the pathogen. The nucleic acid base, purine and pyrimidine directly absorb the photons produced by UV-C radiations which leads to photoproduct formation. Pyrimidine 6–4 pyrimidone photo products (6-4PPs) and cyclobutane pyrimidine dimers

(CPDs) are the major UV-C induced lesions (Friedberg et al., 2006). The replication and transcription of DNA can be interfered if these lesions aren't repaired, which can cause mutation and misreading of the genetic code and eventually cause cell death (Sinha & Häder, 2002).

For more effective and guaranteed results, UV treatment can be used in combination with other novel preservation methods as hurdle technology (Maktabi, Watson & Parton, 2011). When combined with ozone and hydrogen peroxide, UV-C treatment had an increased sporicidal effect. Mild heating, chemical treatment and some other novel technologies are combined with UV-C to improve its lethality towards microorganisms (Jung, Oh & Kang, 2008).

The germicidal action of UV light is strongly dependent on the natural resistance to UV-C of the microorganisms. Shama (2005) has shown that microorganisms differ greatly in the UV doses required for inactivation. Another important factor of survival is the surface on which microorganisms are attached. Gardner and Shama (2000) have shown that surface "topography" plays a major role in determining survival following exposure to UV-C. Microorganisms present on a surface that may be considered smooth are more susceptible to the effects of UV than the microorganisms present on a surface containing crevices inside which they might be shielded from the lethal effects of UV-C. The germicidal effect occurs over a relatively short time that is essentially limited to the time of exposure of the microorganism to the UV source. The exposure times typically range from fractions of a second to tens of seconds (Hui, 2006; Gardner & Shama, 2000).

The UV resistance of a specific microorganism depends on the effectiveness of its DNA repair mechanisms as well as the extent of DNA damage induced by the treatment. In addition, DNA damage and repair mechanisms are affected by different factors, classified as microbial, environmental, and processing factors, which can act before, during, or after UV treatments (López et al., 2005).

Concerning the target microorganism, each organism requires a specific lethal UV dose, thus the microbial characteristics are important for the process efficiency, as sensitivity to UV-C light varies significantly between different types of microorganisms, species, and strains (Koutchma, 2009). Other important factors include microbial cell size, pigment production, irradiation-generated photoproducts, DNA repairability, composition, size, and conformation of the genetic material (Tran & Farid, 2004).

High-intensity UV-C lamps have become available and have a higher potential of destroying surface bacteria on food (Koutchma, 2009). However, UV-C light, being a relatively non-penetrating form of electromagnetic radiation (Koutchma, 2009), limits its application only to surface decontamination of food products. Time from inoculation to UV-C treatment, inoculation level, growth temperature, and inoculation methods could also affect the decontamination efficacy of UV-C light (Stoops et al., 2013).

The surface characteristics of fresh produce is another important factor that needs to be considered when evaluating UV-C decontamination efficacy. It has been well-documented that food surface roughness is correlated with UV-C inactivation efficacy (Adhikari et al., 2015). Due to the surface-level nature of UV-C treatment, its efficacy is hindered by the presence of shadowing effects or the internalization of microorganisms into food tissues. As a result, the presence of local surface roughness or wounded areas is likely to cast shadows over microbial cells, diminishing the germicidal impact of UV-C light (Manzocco & Maifreni, 2011). Inactivation rates were observed to be higher for less hydrophobic fruits with smoother surfaces (apples and pears) as compared to fruits with rougher surfaces (cantaloupe, strawberry and raspberry) (Adhikari et al., 2015). Shadowing occurs when microorganisms absorb the rays and are stacked on top of each other, making it difficult to effectively destroy the organisms in lower layers compared to those in the upper layer. Additionally, when food pieces are treated together, they can cast shadows on one another. As a result, microorganisms located in the upper layers may be inactivated by the UV treatment, but they can shield the remaining microorganisms from its effects (Gomez et al., 2007).

Yaun, Sumner, Eifert, and Marcy (2004), compared the efficacy of UV-C against *E. coli* O157:H7 inoculated onto several fruits and found UV-C was most effective on apple fruit (3.3 log reductions) which had a smoother surface. *Salmonella* populations inoculated onto smooth tomato surfaces decreased by 3.22 logs after UV-C treatment with a dosage of 0.223 kJ m^{-2} (Lim & Harrison, 2016). Similarly, the log reductions of *E. coli* O157:H7 and *Salmonella* due to UV-C exposure to dosage 0.60–6.0 kJ m^{-2} were less when inoculated onto the stem scar area compared to those on the smooth surface of tomato fruit (Mukhopadhyay et al., 2014). A greater UV-C intensity of 3.1 kJ m^{-2} was required for similar inactivation levels of *P. expansum* populations on wounded pear disks (for 2.7 log reduction) compared to intact pear disks (1.7 kJ m^{-2} for 2.8 log reduction) (Syamaladevi et al., 2014).

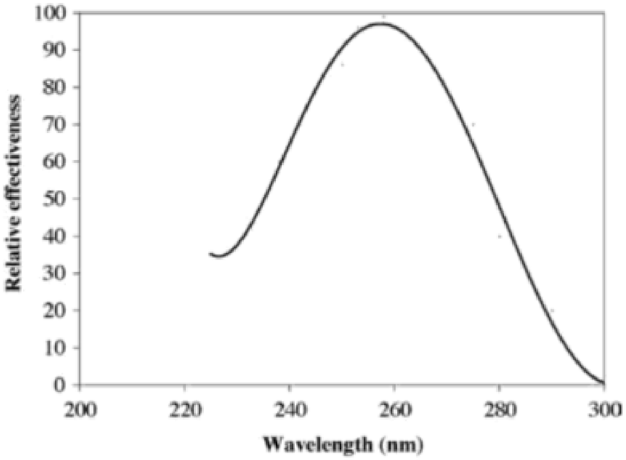


Figure 2.7 Germicidal effect of UV-C related to wavelength
Source: (López et al., 2005).

2.9 Effect of UV treatment on microorganisms

Variations observed among microorganisms, species, and strains have been attributed to different factors, including cell wall thickness, cell size, pigmentation, composition, size, conformation of the genetic material, and DNA repair efficiency. The higher UV resistance of gram-positive versus gram-negative bacteria has been related to the thicker peptidoglycan cell wall of the former, which may hinder the penetration of UV light photons within cells (Beauchamp and Lacroix, 2012). UV resistance varies widely by the type of microorganism, species, and strain. Studies have shown a greater efficiency of UV light in inactivating gram-negative bacteria when compared with gram-positive bacteria, followed by yeast, bacterial spores, fungi, viruses, and protozoa. Protozoa have the highest resistance (López et al., 2005).

Larger-sized cells are more resistant to UV light because there is a higher probability of photons being absorbed by other compounds before affecting DNA. This is one of the reasons molds and yeasts are more resistant than bacteria. In addition, the lower pyrimidine content in the DNA composition of yeast may also contribute to its higher UV resistance (Gayán, Condón and Álvarez, 2014). Cheig et al. (2012) attributed the greater UV-C sensitivity of *E. coli* O157:H7 compared to *L. monocytogenes* to its smaller DNA repair activity since their quantitative analysis of DNA damage showed that the CPD (cyclobutene pyrimidine dimer) formation in *E. coli* O157:H7 was slightly greater than in *L. monocytogenes*. *Listeria monocytogenes* are considered one of the most UV-resistant foodborne pathogens in milk (Lu et al., 2011) and fruit juices (Gabriel and Nakano, 2009). On the other hand, Salmonella spp. are one of the most sensitive genera among seven milk-borne pathogens (Crook et al., 2015).

Different microorganisms typically have different sensitivities to food processing technologies and it applies to UV-C technology too. The UV-C doses needed to reduce 1 log of *E. coli*, *Salmonella Enteritidis* and *L. monocytogenes* populations on fresh-cut broccoli were 1.07, 0.02 and 9.26 kJ m−2, respectively, indicating that *Salmonella Enteritidis* was the most sensitive microorganism to UV-C radiation, while *L. monocytogenes* was the most resistant (Martínez-Hernández et al., 2015). Data on UV effectiveness against the most significant foodborne pathogenic and spoilage bacteria are still limited.

Low doses of UV radiation with a short time of exposure resulted in an effective reduction in the microbial count of fresh-cut spinach during the initial period of storage. UV-C light significantly reduced *L. monocytogenes* growth in fresh-cut spinach for 14 days at 5 °C. During storage, the control reached 4.2- 4.7 log cfu g^{-1}, while UV-C treated leaves increased slightly to 3.6- 4.5 log cfu g^{-1}. After 14 days at 5 °C, leaves treated with 2.4-12 kJ m^{-2} had 3.6- 4.2 log cfu g^{-1} microbial count whereas, for doses 24 and 0 kJ m^{-2}, 4.5 and 4.6 log cfu g^{-1} microbial count were found, respectively. The high dose of 24 kJ m^{-2} likely caused tissue damage, weakening the tissue. This condition allowed for higher counts in the UV-C treatment than the control at the end of the experiment. Other studies have also shown the inability of UV-C to inhibit or reduce pathogen growth in green leaf lettuce (Yaun et al., 2004). During the first 5–8 days, irradiated leaves had lower *S. enterica* and *P. marginalis* counts compared to non-radiated samples. However, these irradiated leaves reached higher counts than the control after 8 days of storage. A low UV-C dose of 2.4 kJ m^{-2} had a similar inhibitory influence on other microbial growth compared to high doses such as 12 or 24 kJ m^{-2} (Yaun et al., 2004).

In case of baby spinach, control leaves reached 5.9 log cfu g^{-1} in the beginning, while leaves treated with 2.4- 12 kJ m^{-2} dosage had 4.4- 4.8 log cfu g^{-1}. At the end of storage of 7 days at 4 °C, no clear differences were obtained among radiated and non-radiated treatments, reaching 8.2- 8.9 log cfu g^{-1}, respectively. It is possible that the use of UV-C radiation on baby spinach leaves caused slight damage to the leaf surface, increasing the nutrient availability for the growth of *Pseudomonas* bacteria. (Escalona et al., 2010).

Populations of pathogenic *E. coli* O157:H7 and *Salmonella* spp. on untreated fruit decreased slowly during storage at 2 and 20 °C; however, populations on fruit treated with 4.4 kJ m^{-2} UV-C decreased rapidly at both temperatures, which suggests that the surface-inoculated bacteria survived poorly following high dose UV-C treatment of apricots (Yun et al., 2013). Pineapple sticks treated with 2 kJ m^{-2} UV-C light, showed slower yeast and lactic acid bacteria growth during 15 days of storage at 6 °C (Manzocco et al., 2016).

Table 2.1 Effect of different UV dosages on the log reduction of various bacteria in different food

UV Dosage	**Food**	**Bacteria**	**Log Reduction**	**Reference**
2.4, 7.2, 12 and 24 kJ m^{-2}	Baby spinach	*Salmonella* CECT 4300 *L. monocytogenes*	0.7–1.6 log CFU/g 1.5–2.2 log CFU/g	Escalona et al., 2010
12–72 kJ m^{-2}	Lettuce	*Salmonella Enteritidis* *Staphylococcus aureus* *Escherichia coli*	1.4 log CFU/g 1.2 log CFU/g 1.7 log CFU/g	Birmpa et al., 2013
2 kJ m^{-2}	Garlic	aerobic microbial population during 30 d post-UV storage at 0 °C.	1 log CFU/g	Park & Kim, 2015.
6 kJ m^{-2}	Fiordilatte cheese	*Pseudomonas* spp. and *Enterobacteriaceae*	1–2 log CFU/g	Lacivita et al., 2016.
0.74 kJ m^{-2}	Apricot	*E. coli O157:H7 and Salmonella spp.*	1–2 log CFU/g	Yun et al., 2013
5 - 25 kJ m^{-2}	Watercress	*E. coli*	1.4–1.7 log CFU/g	Hinojosa et al., 2015
12 - 72 kJ m^{-2}	Strawberry	*Staphylococcus aureus* *E. coli*	1.0 log CFU/g 1.0 log CFU/g	Brimpa et al., 2013
5–40 kJ m^{-2}	Tomato	*L. monocytogenes* *S. aureus*	2.59–3.60 log CFU/g 3.13–3.62 log CFU/g	Sommers et al., 2010
7.56 kJ m^{-2}	Peach	*E. coli*	2.91 log CFU/g	Syamaladevi et al., 2013
7.5 kJ m^{-2}	Pear	*L. monocytogenes* *E. coli*	10 kJ m^{-2} 3.4 log CFU/g	Graça et al., 2017
0.45-3.15 kJ m^{-2}	Mushroom	*E. coli*	0.67–1.13 log CFU/g	Guan at al., 2012

2.10 Effect of UV-C on weight loss

Weight loss in postharvest crops during storage may be attributed to loss of water through transpiration or to loss of substrates (sugar and organic acids) through respiration. Water loss may cause shrivelling of the stored product, with a serious effect on general appearance. Weight loss is an important problem of quality deterioration in horticultural products during postharvest storage (Sharma et al., 2017). The weight loss of vegetables was inhibited when treated with UV during postharvest storage.

The use of UV treatment can result in the formation of a thin and dry film on the surface of fresh-cut apples. This film can potentially help prevent juice leakage and dehydration of the apples during storage. However, it is important to note that the penetration depth of UV-C light is only 0.20 mm, which means that the protective edible film formed would be too thin to be noticeable to consumers when consuming the product (Manzocco et al., 2011). Prolonged exposure to UV-C radiation can have adverse effects on the apple cells. It can lead to the breaking of cell membranes, which in turn may promote the progressive dehydration of the apple slices and contribute to weight loss. Therefore, it becomes evident that the application of UV treatment should be done at a mild intensity to minimize these negative effects. By using UV-C at lower intensities, it is possible to reduce weight loss and inhibit the browning of fresh-cut apples, thus preserving their quality during storage

In addition, UV treatment also inhibited weight loss of fruits during postharvest storage. UV-A, UV-B, and UV-C treatment with 6.0 kJ m^{-2} reduced the weight loss of blueberry fruits during the late postharvest storage (Nguyen et al., 2014). Similarly, 4.0 kJ m^{-2} dosage was also able to significantly reduced weight loss in blueberries for 8 days at 4 °C (Xu et al., 2016).

Non-irradiated strawberry fruit lost around 6 and 11% of fresh weight after 3 and 5 days at 10 °C and similar values were found in fruit irradiated with low intensity of 3 Wm-2 UV-C dosage of 4 KJm^{-2}. The treatment performed at the highest intensity of 33 Wm^{-2} significantly reduced weight loss. In tomato, after 4 days of storage at 20 °C, no differences were found between the control and treated fruit regardless of the UV intensity. In contrast, after 9 days at 20 °C, fruit irradiated with high UV-C fluency of 33 Wm^{-2} showed lower weight loss than the control (Cote et al., 2013).

A high UV dose of 219 kJ m^{-2} treatment reduced weight loss in Apple (Assumpção et al., 2018) and a dosage of 1.7 kJ m^{-2} reduced the weight loss of vegetable amaranth and maintained high levels of hemicellulose and carotenoids during shelf life at 20 °C (Gogo et al., 2017). In bell pepper, a combination of UV treatment and hot water dipping resulted in lower weight loss than in the control (Karasahin et al., 2006). A modest weight loss was also noted in UV-treated sweet potatoes (Charles & Arul, 2007), but weight loss was higher in UV-treated tomato fruits than in the untreated control, but not significant enough to cause shrivelling (Maharaj et al., 1999).

2.11 Effect of UV-C on colour

The colour and general appearance (turgidity or shrivelling) of fresh stored fruits and vegetables can be a decisive factor in their acceptance or rejection by the consumer. During ripening, fruit colour change is due to chlorophyll loss, the unveiling of pre-existing carotenoids, and the synthesis of carotenoids such as lycopene and other pigments such as anthocyanins. From the standpoint of prolonging shelf-life, it is desirable that these changes take place at a slower rate. Several authors have described the delay in colour change induced by UV-C in treated fruits and vegetables (Charles & Arul, 2007).

The loss of green and yellowing caused by the degradation of chlorophyll is a major quality deterioration problem for some leafy vegetables and fruits during postharvest storage. UV-C treatment led to the maintenance of the green colour during postharvest storage. UV-C light exposure can lead to the inactivation of various enzymes, including polyphenol oxidase (PPO). Research has demonstrated that apple slices subjected to higher UV-C light fluence (24.0 kJ/m^2) exhibited a significantly higher b* value compared to samples treated for shorter durations. This finding indicates that prolonged exposure to UV-C light reduces the effectiveness of the treatment in inhibiting enzymatic browning. This reduction in efficacy can be attributed to the breakage of cellular membranes, resulting in a loss of cell compartmentalization. As a consequence, there is increased contact between the enzyme and substrate, counteracting the denaturation of PPO by UV-C light (Manzocco & Quarta, 2009; Manzocco et al., 2011).

Multiple cycles of UV-C treatment with 2.46 kJ m^{-2} delayed the degradation of chlorophyll of leaf vegetables like leek, spinach and cabbage for 5 days at 4 °C (Liao et al., 2016). Similarly, UV-C treatment with 3.4 kJ m^{-2} maintained higher chlorophyll-*a* content during the storage of vegetables at low temperatures at 5 °C. However, at room temperature, the same dose of UV-C treatment had no inhibitory effect on the degradation of chlorophyll-*a* (Gogo et al., 2017).

In strawberries, the lightness (L*) decreased along with surface colour saturation (chroma) as storage time progressed. UV-C treatment delayed the darkening of the fruit. However, this colour change was slowed down by exposure to high UV-C intensity and no difference was observed between control and low UV-C intensity-irradiated fruit (Cote et al., 2013). Low-dose UV treatment of 2.0 kJ m^{-2} and 4.0 kJ m^{-2} maintained higher Hue values and inhibited the increase of lightness and chlorophyll degradation for 17 days during the initial phase at 0 °C (Darré et al., 2017). In tomatoes, high-dose UV treatment with 20 kJ m^{-2} and 40 kJ m^{-2} maintained L* values and inhibited the increase of Hunter values (L*, a* b*) for 37 days during storage at 14 °C (Liu et al., 2011). In strawberries, UV-C treatment with 4.1 kJ m^{-2} maintained better fruit colour for 4 days at 20 °C (Li et al., 2014). In the case of lime, treatment with 19 kJ m^{-2} maintained a higher hue value and inhibited the degradation of chlorophyll for 30 days at 25 °C (Kaewsuksaeng et al., 2011). In broccoli florets, low-dose of 2.0 kJ m^{-2} and 4.0 kJ m^{-2}, maintained higher Hue values, and inhibited increase of lightness and chlorophyll degradation for 17 days during the initial phase at 0 °C (Darré et al., 2017).

In pepper (Vicente et al., 2005), broccoli (Costa et al., 2006) and tomato (Liu et al., 1993), UV-C irradiation significantly delayed chlorophyll loss. However, colour development was accentuated by UV treatment in strawberry fruits with an associated increase in anthocyanin accumulation (Baka et al., 1999). Doses higher than the hormetic dose led to detrimental bronzing and duller appearance in tomato fruits and altered the appearance of citrus fruits. Fruit surface scalding occurred in papaya at all UV doses applied. At higher doses, yellow lemon fruits exhibited less damage than green fruits (Ben-Yehoshua et al., 1992).

2.12 Effect of UV treatment on Sensory attributes

Ultraviolet light treatment has been proven to be an effective inhibitory treatment for postharvest senescence of fruits and vegetables in recent years, it can extend the shelf life of postharvest fruits and vegetables, maintain the quality during storage of postharvest fruits and vegetables, including delaying the degradation of chlorophyll in green fruits and vegetables and reducing the chilling damage caused by cold storage of fruits (Zhang & Jiang, 2019).

UV-C treatment of *Agaricus bisporus* with 1.0 kJ m^{-2}, maintained high visual quality in the first 21 days at 4 °C by inhibiting the degree of browning (Lei et al., 2018). UV-C dosage of 4.0 kJ m^{-2} significantly reduced the decay rate and maintained high hardness in blueberries for 8 days at 4 °C (Xu et al., 2016). Garlic treated with a UV-C dosage of 2.0 kJ m^{-2} maintained high hardness and colour, increased total phenolic content, and reduced microbial communities for 15 days at 0 °C (Park & Kim, 2015). High UV-B dosages of 20 kJ m^{-2} and 40 kJ m^{-2} resulted in high hardness in tomatoes for 37 days and in storage at 14 °C (Liu et al., 2011). High hardness was observed in peaches treated with UV-C 3.0 kJ m^{-2} (Yang et al., 2014); mangoes treated with 5 kJ m^{-2} for 5 days at 6 °C (Ruan et al., 2015); strawberries treated with 4.1 kJ m^{-2} for 4 days at 20 °C (Li et al., 2014); and cherry tomato treated with 4.2 kJ m^{-2} for 35 days at 18 °C (Bu, Aisikaer & Ying, 2013).

Multiple cycles of UV-C of 2.46 kJ m^{-2} on leafy vegetables like leek, spinach and cabbage, delayed the degradation of chlorophyll for 5 days at 4 °C. (Liao et al., 2016). Cyclic low-dose UV-C treatment with 4.0 kJ m^{-2}, more effectively reduced weight loss, and decay rate, maintained better colour and firmness, increased the respiration rate in the early stage of storage, and delayed the ageing process of strawberries for 13 days at 0 °C (Araque et al., 2018). UV-C treatment of pineapple with 26.4 kJ m^{-2} reduced the decay rate and maintained better colour characteristics in the peel for 28 d at 10 °C (Sari, Setha & Naradisorn, 2016).

The extensive loss of sensory quality can be due to overexposure to UV-C dosage. Similar quality losses were also observed in other crops treated with doses higher than the hormetic dose. UV-C was not considered to be an effective method by D’hallewin et al. when compared with heat treatment, because of the poor visual appearance of the UV-C-treated fruits, even though the level of decay control was significant (less than 50% of the control) (D'hallewin et al., 1993).

2.13 Effect of UV-C on firmness

Firmness is an important quality parameter of the fruits, as the fruit senescence, the fruit will become severely softened (Yang et al., 2014). Firmness is a very important quality factor for postharvest fruits and vegetables. Firmer produce is better able to withstand postharvest handling and transportation. UV-C irradiation appears to slow down the softening rate of several fleshy fruits. Higher firmness indices were reported for UV-treated strawberries (Baka, 2000), peaches (Charles & Arul, 2007), apples (Lu et al., 1991) and pepper (Vicente et al., 2005). Similar results were observed for UV-treated tomatoes, which remained firmer over a longer period compared with the control (Charles & Arul, 2007).

Firmness decreased almost 50% in control strawberries after 3 days of storage at 10 °C. Softening was significantly reduced in UV-C-treated fruit. High UV radiation intensity was more efficient to prevent softening. During the first 3 days of storage, no changes in firmness were detected in high UV-C intensity-treated fruit and even after 5 days, the UV-C-treated fruit remained firmer than the control. In tomatoes, there was a slight reduction of softening by UV-C treatments but no differences resulted from changing the radiation intensity (Cote et al., 2013).

Cell wall degradation directly leads to softening of the fruit during storage. Polygalacturonase (PG), and pectin methylesterase (PME) are the major degrading enzymes of protopectin in cell walls (Zhang & Jiang, 2019). Activities of cell wall degrading enzymes such as PME and PG are inhibited by UV-C treatment. It has been reported that UV-C treatment (4.2 kJ m^{-2}) significantly reduced the activity of PG and PME of cherry tomatoes, maintained high acid-soluble pectin and cellulose content and suppressed the expression of related genes during postharvest storage (Bu et al., 2013). In UV-treated tomatoes, the activity of polygalacturonase was lower than in the control (Stevens et al., 2004). The activity of other cell-wall-degrading enzymes was also found to be reduced in UV-treated tomatoes (Barka et al., 2000). UV-C treatment (2.0–4.1 kJ m^{-2}) effectively maintained the higher firmness of fruits and vegetables during storage, including garlic, blueberry, strawberry, peach, and cherry tomato (Zhang & Jiang, 2019).

To investigate the effectiveness of the UV-C light treatment on the inactivation of microorganisms and its effect on the shelf life of fresh ready-to-eat (RTE) salad leafy greens, a comprehensive materials and methods approach was employed in this study.

3.0 Materials and Methods

3.1 Description of overall experimental Design

The efficacy of UV-C light on the inactivation of microorganisms present on the surfaces of four fresh ready-to-eat (RTE) leafy greens was investigated. The UV-C treatment of sanitised and unsanitised leafy greens was carried out in three phases.

The objective of Phase I was to determine the optimum UV-C dosage for microbial reduction on spinach, kale, mesclun and rocket with minimal changes in the overall visual appearance of the leaves. The sanitised salad was supplied by a local supplier in Auckland, New Zealand, and the unsanitised samples were purchased from the local supermarket. In phase I, sanitised and unsanitised samples were subjected to UV-C doses ranging from 100-700 mJ/cm^2 followed by aerobic plate counts (APCs) of the UV-C treated and untreated samples. The fresh leafy greens used in Phase 1 were purchased from a local supermarket in Auckland, New Zealand, and they belonged to the same batches.

In Phase II, the effect of the two optimum doses obtained from Phase 1 for reducing surface microflora was studied on the APCs of sanitised and unsanitised spinach, kale, mesclun and rocket. The best dosage was selected for further studies. The fresh leafy greens used in Phase II were supplied by a commercial company in New Zealand. The experiments used 2^3 designs.

The third Phase of the study investigated the effect of selected UV-C dosage on the shelf-life of the leafy greens during refrigerated storage. The UV-C treated, and untreated samples were analysed for their physiological, microbial and sensory attributes during storage for 12 days/4 °C. The data obtained from APCs and Hunter L*, a*, b* values were analysed by the analysis of variance (ANOVA) using the General Linear Model and significant means were separated by the Tukey's Test at level 5% (Minitab Inc., Pennsylvania, USA).

3.2 Phase I

In phase, I of the study, dosages ranging from 100 to 700 mJ/cm^2 with an increment of 50 mJ/ cm^2 were applied to the salads to determine their effects on different salads and select the optimum irradiation energies for application in phase II. The exposure time for the treatment varied from 4-20 minutes based on the dosage.

3.2.1 Salad sample collection

Sanitised and unsanitised samples of RTE spinach, kale, rocket and mesclun were purchased from and obtained from a local supplier in New Zealand. The samples consisted of leaves detached from the stem. Leaves with defects such as yellowing, decay, cuts and bruising were discarded.

3.2.2 UV-C treatment of salad samples

The JouleSafe 21A0043 disinfection unit (Figure 3.1) Radiant Industrial Solutions) was used for the UV-C surface irradiation of the salad leafy greens.

Figure 3.1 JouleSafe 21A0043

Stainless-steel trays made with UV-safe material were disinfected using ethanol (70%). Then, 10g of sanitised and unsanitised salads were placed on the trays as shown in Figure 3.2. The leaves were handled using stainless-steel tweezers sterilised using 70% ethanol.

Figure 3.2 salad samples arranged on UV safe stainless-steel trays

Then the samples were irradiated with germicidal emitting lamps with 360-degree exposure. The sanitised and unsanitised samples of RTE spinach, kale, rocket and mesclun, were subjected to UV-C doses ranging from 100-700 mJ/cm^2. The time of exposure varied from 4-20 minutes based on the dosage.

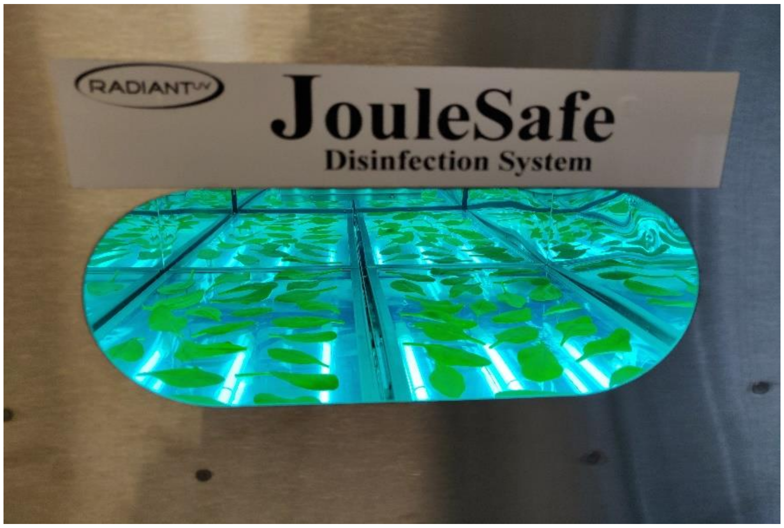


Figure 3.3 Image of UV-C treatment of salad leaves

Two groups of treatments (sanitised and unsanitised) were treated with UV-C doses as shown in Figure 3.3.

3.2.3 Microbiological analysis

The UV-C treated samples and untreated samples (~10 g) were homogenized in 90 mL sterile peptone water (Oxoid™ Peptone) for 1 min in a sterile stomacher bag using a masticator and 1ml of the solution was aseptically transferred into 9 ml of peptone water to prepare serial dilutions up to 10^{-8} (spinach), 10^{-5} (kale), 10^{-7} (rocket) and 10^{-8} (mesclun). The diluted samples were pour-plated on aerobic plate count agar (Oxoid^{TM}, ThermoFisher, New Zealand) and incubated at 35° C/48 hours. The results of the incubated plates were used to determine the microbial log CFU/cm^2 reductions following UV-C treatment. Microbial analysis was done in duplicate, and the experiment was replicated twice. The results were expressed as log CFU/cm^2 (Abdussamad, Rasco, & Sablani, 2016; Gogo et al., 2017).

3.2.4 Standardization of UV-C dose

The effect of various ranges of UV-C doses from 100-700 mJ/cm^2 was studied and an optimum dose with a minimum exposure time, maximum microbial reduction and least physical changes to the sanitised and unsanitised leafy greens were selected. The effect of UV-C treatment was studied along with the control to understand the microbial reduction effect (Allende, Tomás-Barberán, & Gil, 2006).

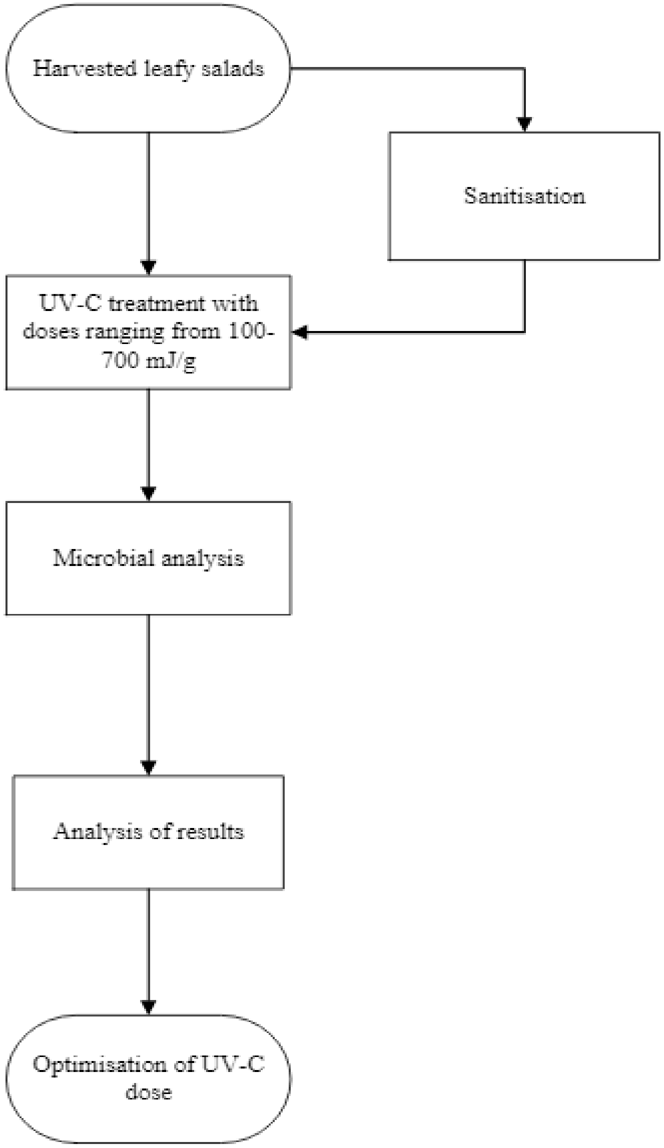


Figure 3.4 Overview of Phase I experiment

3.3 Phase II

A multilevel factorial design with 3 factors (UV-C dosage, type of salad, sanitization) with 4 levels was generated to determine the optimum UV-C dosage for the surface pasteurization of sanitised and unsanitised spinach, kale mesclun and rocket as shown in Table 3.1.

Table 3.1 Randomized factorial design generated by Minitab Inc. (2021) for the optimization of UV-C dosage on microbial reduction

StdOrder	RunOrder	PtType	Blocks	Leaf Type	Sanitised	UV-C Dosage
31	1	1	2	Kale	No	0
35	2	1	2	Kale	Yes	100
37	3	1	2	Mesclun	No	0
45	4	1	2	Rocket	No	150
26	5	1	2	Spinach	No	100
48	6	1	2	Rocket	Yes	150
40	7	1	2	Mesclun	Yes	0
39	8	1	2	Mesclun	No	150
33	9	1	2	Kale	No	150
34	10	1	2	Kale	Yes	0
44	11	1	2	Rocket	No	100
29	12	1	2	Spinach	Yes	100
32	13	1	2	Kale	No	100
27	14	1	2	Spinach	No	150
25	15	1	2	Spinach	No	0
30	16	1	2	Spinach	Yes	150
41	17	1	2	Mesclun	Yes	100
43	18	1	2	Rocket	No	0
38	19	1	2	Mesclun	No	100
28	20	1	2	Spinach	Yes	0
47	21	1	2	Rocket	Yes	100
36	22	1	2	Kale	Yes	150
46	23	1	2	Rocket	Yes	0
42	24	1	2	Mesclun	Yes	150
21	25	1	1	Rocket	No	150
15	26	1	1	Mesclun	No	150
5	27	1	1	Spinach	Yes	100
24	28	1	1	Rocket	Yes	150
20	29	1	1	Rocket	No	100
14	30	1	1	Mesclun	No	100
17	31	1	1	Mesclun	Yes	100
16	32	1	1	Mesclun	Yes	0
18	33	1	1	Mesclun	Yes	150
2	34	1	1	Spinach	No	100
1	35	1	1	Spinach	No	0
13	36	1	1	Mesclun	No	0
12	37	1	1	Kale	Yes	150
8	38	1	1	Kale	No	100
19	39	1	1	Rocket	No	0
3	40	1	1	Spinach	No	150
10	41	1	1	Kale	Yes	0
6	42	1	1	Spinach	Yes	150
22	43	1	1	Rocket	Yes	0
4	44	1	1	Spinach	Yes	0
23	45	1	1	Rocket	Yes	100
11	46	1	1	Kale	Yes	100

9	47	1	1	Kale	No	150
7	48	1	1	Kale	No	0

3.3.1 Description of the factorial design

A general full factorial multilevel design consisting of 3 factors (Table 3.2) was generated (α = 0.05) (Table 3.1) to determine the optimum UV-C dosage for the surface pasteurization of sanitised and unsanitised spinach, kale mesclun and rocket.

Table 3.2 Factors used to create the general factorial design

Factor	Factor Name
A	Salad type
B	Sanitisation
C	UV-C dosage (mJ/cm^2)

Note
Salad type (A): spinach; kale; mesclun; rocket
Sanitisation (B): yes/no
UV-C dosage (mJ/cm^2) (C): 0; 100; 150 mJ/cm^2

Industrially sanitised and unsanitised RTE samples of spinach, kale, rocket and mesclun were supplied by a local supplier in New Zealand. The samples were treated with UV-C dosages of 100, 150 mJ/cm^2 and controls (0 dosage) were included.

Suitable dilutions of the samples were prepared to enumerate aerobic plate counts. 1 mL of the dilutions were pour-plated using plate count agar and were incubated at 35^o C for 48 hours. The grown colonies were enumerated and expressed as log CFU cm^{-2} (Abdussamad, Rasco, & Sablani, 2016; Gogo et al., 2017). The analysis was done in duplicate the experiment was replicated twice.

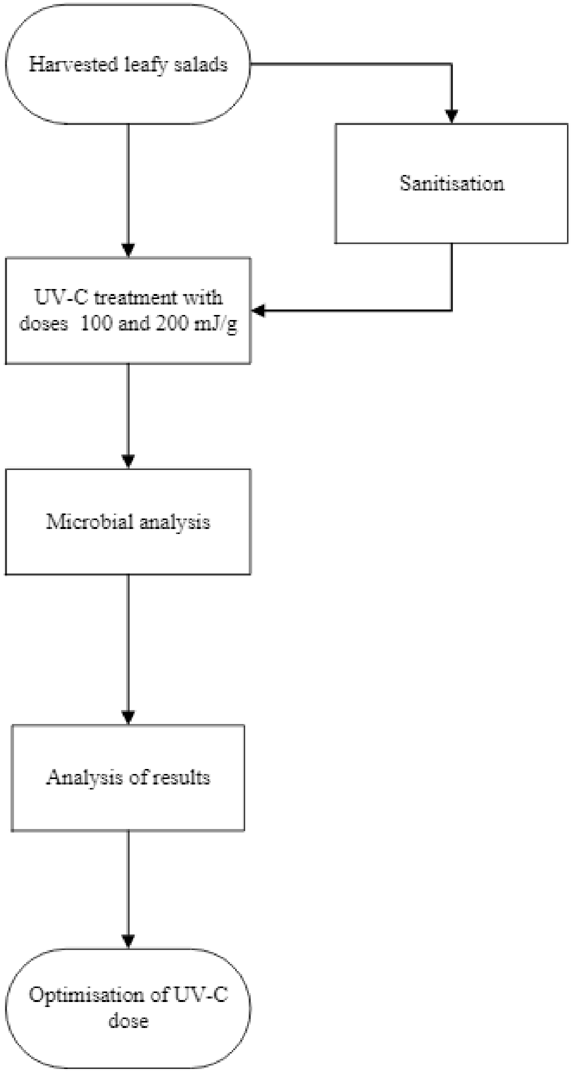


Figure 3.5 Outline of the protocol of phase II

Note: sanitised = industrially sanitised; APC = aerobic plate count

3.4 Phase III

In the final phase III, the effect of the selected UV-C dosage obtained from phase II was studied on various factors like microbial growth, sensory properties, weight loss, colour and overall acceptability during storage for 12 days at 4 ˚C.

3.4.1 Packaging and Storage of salad samples

The optimum UV-C dosage of 100 mJ/cm^2 was selected in phase II and this dose (UV-C) was used to treat the sanitised and unsanitised leafy salad samples of spinach, kale, rocket and mesclun. The UV-C treated and UV-C untreated samples of sanitised and unsanitised leafy salads were packaged in microperforated plastic packages and the packages were heat-sealed. The experiment was carried out in duplicate. Microbial, physiological, and sensory analyses were conducted on 0, 4, 8 and 12 days of storage at 4 ˚C (Martínez-Hernández., 2015).

3.4.2 Microbial Analysis

The enumeration of APC of the UV-C treated and untreated samples of sanitised and unsanitised salads were conducted during storage for 12 days (4 ˚C). The analysis of APC was prepared as described previously in section 3.2.3. Samples were analysed on days 0 (fresh), 4, 8 and 12 during storage (4 ˚C). Presumptive colonies of *L. monocytogenes,* coliforms, *S. aureus* and *Salmonella* spp. were also enumerated. The pathogens were enumerated by spread plating except for coliforms on selective media using the manufacturer's instructions.

L. monocytogenes was analysed using the Oxford agar (Oxoid, UK), *S. aureus* (Oxoid, UK) was analysed using Baird-Parker agar (BPA, Oxoid, UK), and *Salmonella* spp. was analysed using Xylose-Lysine-Desoxycholate (XLD, Thermo Scientific™ Oxoid™) agar. From the dilutions 10^{-1}, 10^{-2} and 10^{-3}, 0.1 mL was spread on the surface of respective pre-poured media using a sterile disposable plastic spreader. The plates were incubated at 37°C for 24–48 hours (Hunt et al., 2017 & Kumar et al., 2016). Coliforms were analysed using the pour plate technique with appropriate dilutions on Violet Red Bile agar (VRBA) with an overlay of approximately 5 mL of VRBA agar (Van et al., 2012).

The microbial counts were performed in duplicate on the processing day and on days 4, 8 and 12 of storage at 4ºC and expressed as log CFU/cm^2 (Martínez-Hernández et al., 2015).

3.4.3 Weight Loss

Weight loss was determined by weighing the samples before and after the storage period. The weight loss was calculated as a percentage of weight loss on day 12 of storage based on the initial weight of the samples on day 0 (Manzocco et al., 2011). The loss of weight of fresh samples was determined as shown in equation x (Gogo et al., 2017).

$$\% \text{ Weight loss} = \frac{(Wi - Wf) * 100}{Wi} \dots\dots\dots\dots\dots.. \text{equation } 1$$

Wi = initial weight of sample
Wf = final weight of sample

3.4.4 Colour

The colour of the sanitised and unsanitised samples was measured using a colorimeter equipped with a Minolta CR-300 (Minolta, Japan) measuring head. The instrument was standardised against a white tile before measurements according to the manufacturer's instructions (Minolta, Japan). The colour was expressed in L*, a* and b* Hunter scale parameters, where L* represents the lightness, a* represents the greenness and b* represents the yellowness of the salad samples (Manzocco et al., 2011). The colour was measured on days 0, 4, 8 and 12 for each sample.

3.4.5 Sensory Analysis

A focus group sensory evaluation was conducted with 8-10 participants to evaluate the sensory characteristics of fresh and stored salad samples. Ethics approval was obtained for the sensory analysis (Ethics Notification Number: 4000022998). The samples were evaluated based on the appearance, flavour, texture, odour overall acceptability of the UV-C treated and untreated samples of sanitised and unsanitised salads at fresh on day 0, then during refrigerated storage (4°C) on day 4, 8 and 12 (Allende et al., 2006).

3.4.6 Statistical Analysis

The effect of various UV-C doses on the treatment of leafy salads was analysed using the General multi-factorial design of Minitab. Analysis of variance (ANOVA) was performed to determine any significant difference in the microbial cell counts, Hunter L*, a*, and b* values at $P<0.05$ level (Abdussamad, Rasco, & Sablani, 2016).

The results of this analysis were shown by using the Pareto charts of standardised effects. The Pareto chart is employed for the evaluation of results from experimental designs. These charts, commonly utilized in evaluating experimental designs, present the effects of factors and their interactions as standardized effects (Ferreira et al., 2018). The standardized effects serve as a means to test the null hypothesis, which assumes that the effect is zero. By employing a Pareto plot, one can identify the most crucial factor and interaction effects in relation to the results. The plot showcases the absolute values of these effects and incorporates a reference line. Effects that surpass this reference line are considered statistically significant (Antony, 2023).

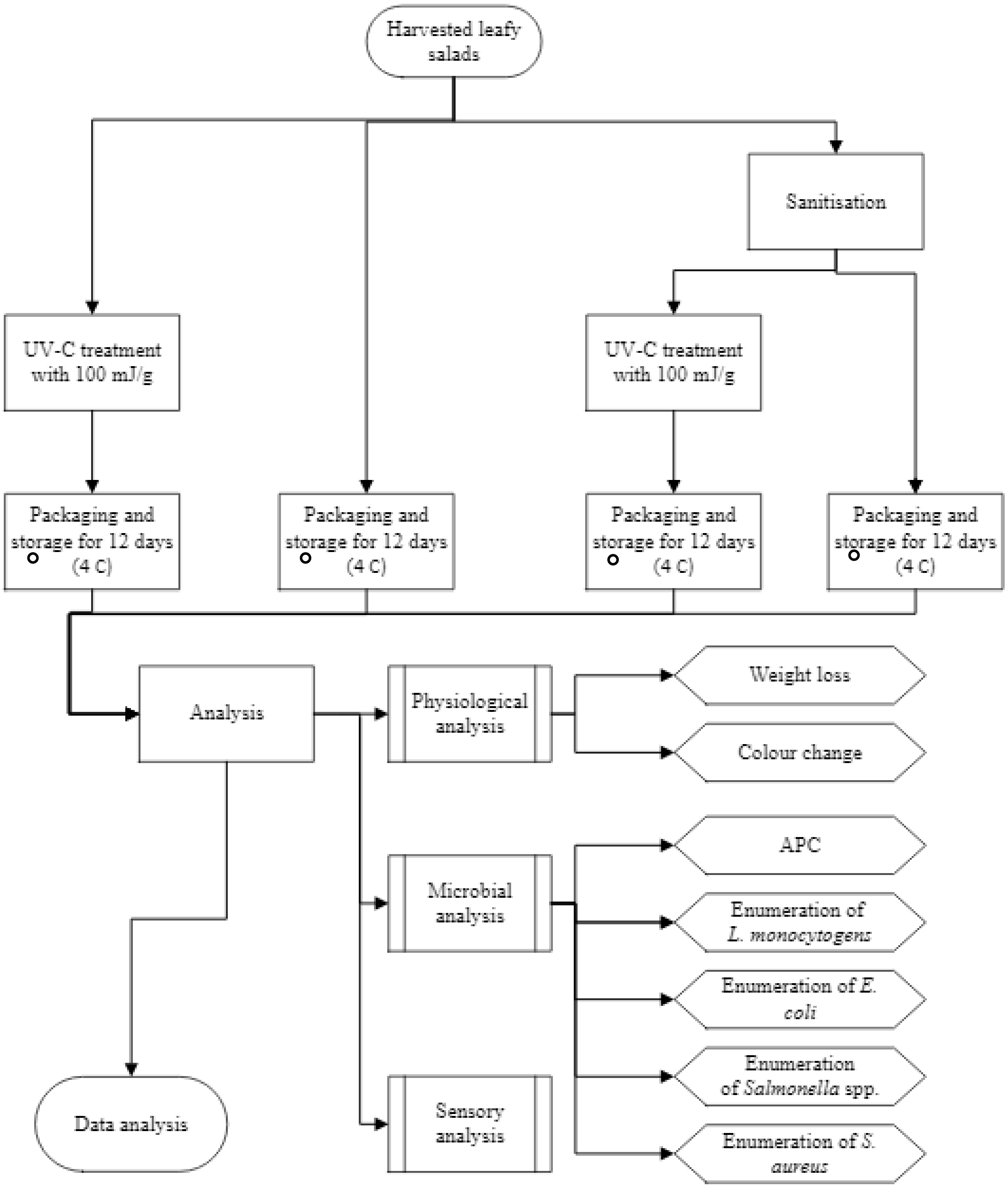


Figure 3.6 Overview of experiments conducted in Phase III

Note: Sanitisation =industrially sanitised

4.0 RESULTS AND DISCUSSION

4.1 Phase I - Effect of UV-C dosage ranging from 100-700 mJ/cm^2

4.1.1 Sanitised and Unsanitised Spinach

Table 4.1 Microbial log reductions (log CFU/cm^2) of sanitised and unsanitised spinach after UV-C treatment

Trial	**Dosage mJ/cm^2**	**Unsanitised Spinach**		**Sanitised Spinach**		**Visual observations**
		Initial mean after treatment log CFU/cm^2	**Log Reduction log CFU/cm^2**	**Initial mean after treatment log CFU/cm^2**	**Log Reduction log CFU/cm^2**	
1	**0**	***7.48 ± 0.02**		***6.06 ± 0.78**		Fresh, green, firm texture, smooth glossy surface
	100	6.01 ± 0.14	1.46	5.25 ± 0.03	0.81	Free from discolouration and damage with no visible dehydration
	300	6.17 ± 0.47	1.30	4.90 ± 0.08	1.16	Partially dehydrated and loose texture
	400	5.13 ± 0.34	2.34	4.89 ± 0.07	1.18	Highly fragile, wilted and dehydrated
	500	5.69 ± 0.02	1.78	4.48 ± 0.06	1.59	Highly dehydrated, sticky and shrivelled
2	**0**	***5.80 ± 0.23**		***6.12 ± 0.00**		Fresh, healthy, good conditioned leaves
	100	4.07 ± 0.05	1.73	4.07 ± 0.06	2.05	Undamaged leaves with no discolouration and visible dehydration
	150	3.47 ± 0.09	2.33	4.33 ± 0.43	1.78	Free from mechanical damage or damage by any other means
	200	3.99 ± 0.16	1.80	4.76 ± 0.01	1.36	Partially dehydrated and sticky texture
	400	3.80 ± 0.22	2.00	3.99 ± 0.03	2.13	Sticky, shrivelled, dehydrated leaves
3	**0**	**-**	**-**	***6.03 ± 0.00**		Fresh, healthy, good in appearance
	100	**-**	**-**	4.42 ± 0.16	1.75	Similar to fresh, untreated leaves
	150	**-**	**-**	4.21 ± 0.02	1.82	No dehydration and shrivelling
4	**0**	***6.22 ± 0.18**		***6.41 ± 0.64**		Healthy leaves with no visible damage
	100	4.94 ± 0.27	1.28	4.69 ± 0.25	1.71	Similar to fresh leaves with no visible dehydration
	150	4.77 ± 0.73	1.45	4.09 ± 0.08	2.31	No dehydration and shrivelling
5	**0**	***7.11 ± 0.01**		***6.13 ± 0.09**		Fresh green leaves with steady structure
	100	4.81 ± 0.00	2.30	3.82 ± 0.10	2.31	No visible dehydration and injuries
	150	5.07 ± 0.06	2.04	4.15 ± 0.00	1.97	Appearance similar to the non- UV treated samples

Notes:

- n (sample size) = 2;
- Log reduction = (initial mean of control) – (initial mean of dosage)
- (±) Standard deviation of the mean of two samples
- (-) Data not available

In the initial trial involving spinach, the total APC for the unsanitized control sample was 7.48 ± 0.02 log CFU/cm², while the sanitized control exhibited a count of 6.06 ± 0.78 log CFU/cm². The microbial log reduction across dosages of 100 to 500 mJ/cm² ranged from 1.30 to 2.34 log CFU/cm² for unsanitized spinach and from 0.81 to 1.59 log CFU/cm² for sanitized spinach. The highest log reduction of 2.34 log CFU/cm² occurred at a UV-C dosage of 400 mJ/cm². Notably, a significant microbial reduction was achieved at 500 mJ/cm², but it resulted in dehydration and damage to the spinach leaves. Similarly, Guerrero-Beltr and Barbosa-C (2004) also observed dehydration and damage at higher doses. The results indicated that a UV-C dosage of 100 mJ/cm² was preferable for unsanitized spinach, producing a microbial reduction of 1.46 log CFU/cm² without causing visible leaf damage.

In the second trial, lower doses of 100, 150, 200, and 400 were selected. It was noted that even at a dosage of 200 mJ/cm², the spinach leaves suffered dehydration. Microbial log reductions of 2.05 and 1.78 log CFU/cm² were observed for sanitized samples, and 1.73 and 2.33 log CFU/cm² for unsanitized samples, at UV doses of 100 and 150 mJ/cm², respectively, without causing leaf damage and preserving quality.

Moving to the third trial, the sanitized spinach samples were exposed to UV-C dosages of 100 and 150 mJ/cm². The total colony forming units for the sanitized control leaves were 6.02 log CFU/cm². Substantial reductions of 1.75 and 1.82 log CFU/cm² in microbial count were observed for UV-C doses of 100 and 150 mJ/cm², respectively, in sanitized spinach samples.

In the fourth trial for spinach, the total CFU/cm² for the unsanitized control sample was 6.22 ± 0.18 log CFU/cm². Maximum log reductions of 2.31 and 1.45 CFU/cm² were observed at a UV dose of 150 mJ/cm² for sanitized and unsanitized spinach, respectively. Substantial microbial reduction was also noted for both 100 and 150 mJ/cm². In the fifth trial, spinach samples were treated with doses of 100 and 150 mJ/cm². The total plate counts for the unsanitized and sanitized control spinach samples were 7.11 ± 0.01 and 6.13 ± 0.09 log CFU/cm², respectively. Table 4.1 illustrates the significant microbial log reduction observed for both doses. Additionally, it was observed that doses exceeding 200 mJ/cm² resulted in leaf damage and dehydration. The series of trials confirmed that doses of 100 and 150 mJ/cm² were ideal for baby spinach leaves, providing effective microbial log reduction without compromising the physical appearance of the leaves.

4.1.2 Sanitised and Unsanitised Kale

Table 4.2 Microbial log reductions (log CFU/cm^2) of sanitised and unsanitised kale after the UV-C treatment

Trial	**Dosage mJ/cm^2**	**Unsanitised Kale**		**Sanitised Kale**		**Visual observations**
		Initial mean after treatment log CFU/cm^2	**Log Reduction log CFU/cm^2**	**Initial mean after treatment log CFU/cm^2**	**Log Reduction log CFU/cm^2**	
1	**0**	***8.10**		***6.92**		Healthy leaves with rough and hard texture
	100	8.01	0.09	5.79	1.12	Firm structured leaves with no visible dehydration
	300	7.51	0.58	7.06	0.14	Firm leaves free from injuries and discolouration
	500	7.54	0.56	6.01	0.91	Wilted, dehydrated leaves with loose structure
	700	6.47	1.63	6.02	0.89	Highly dehydrated, loose structured and sticky leaves
2	**0**	***6.17 ± 0.09**		***4.80 ± 0.74**		Leaves with stable structure and rough texture
	100	4.18 ± 0.54	1.19	3.61 ± 0.47	1.99	Steady structure and no visible dehydration
	150	4.51 ± 0.61	2.11	2.69 ± 0.99	1.66	No changes in the appearance or the texture of the leaves
	200	4.89 ± 0.13	0.98	3.82 ± 0.21	1.28	No wilting, visible dehydration and colour change
	300	3.34 ± 0.91	1.48	3.33 ± 0.68	2.82	Slight dehydration but firm leaves
3	**0**	***6.11 ± 0.01**		***6.13 ± 0.05**		Good physical appearance and no damage. The unsanitised kale was curled up and had rough surface as compared to sanitised baby kale.
	100	4.96 ± 0.03	1.14	3.99 ± 0.03	2.14	No visible change in the appearance of the leaves
	150	5.56 ± 0.08	0.55	4.06 ± 0.01	2.06	Firm and stable leaves
	200	5.84 ± 0.06	0.26	4.10 ± 0.06	2.03	No noticeable dehydration or changes observed in the leaves
	300	5.70 ± 0.07	0.41	4.14 ± 0.02	1.99	A little dehydration was observed, making the leaves a little soft.

Note:

- **Bold* =** non-UV-C treated control kale sample
- n (sample size) = 2;
- Log reduction= (initial mean of control) – (initial mean of dosage)
- (±) Standard deviation of the mean of two samples

In the initial trial involving kale, it was observed that the initial total counts for unsanitized and sanitized kale were 8.10 and 6.92 log CFU/cm² respectively. A significant microbial log reduction was noted at 700 mJ/cm² for unsanitized kale, while for the sanitized samples, a high reduction of 1.12 log CFU/cm² was observed at 100 mJ/cm². Although reasonable results were obtained at 700 mJ/cm², this dosage was not preferred due to the considerable dehydration and shriveling observed in the leaves.

In the second trial, the total counts for unsanitized and sanitized kale were 6.17 ± 0.09 log CFU/cm² and 4.80 ± 0.54 log CFU/cm² respectively. A microbial log reduction of 2.11 log CFU/cm² was observed for unsanitized kale at 150 mJ/cm², and for the sanitized samples, a substantial reduction of 2.82 log CFU/cm² was achieved at 300 mJ/cm². Additionally, a significant reduction of 1.99 log CFU/cm² for sanitized kale was observed at the 100 mJ/cm² dose.

In the third kale trial, the initial microbial counts for the control were 6.11 ± 0.01 and 6.13 ± 0.05 log CFU/cm² for unsanitized and sanitized kale, respectively. The microbial log reduction for sanitized kale across doses ranging from 100 to 300 mJ/cm² ranged from 1.99 to 2.14 log CFU/cm², while for unsanitized kale, it ranged from 0.26 to 1.14 log CFU/cm². Notably, in the case of sanitized baby kale, the most significant reductions were achieved at doses of 100 and 150 mJ/cm². However, for unsanitized kale, a substantial reduction of 1.14 log CFU/cm² was noted only at 100 mJ/cm², and no marked reductions were observed for the other doses. The curled and irregular structure of the locally sourced kale could have impeded the penetration of UV-C radiation to the surface, potentially providing shelter to microbes present on the kale's surface. Manzocco & Maifreni (2011), also found diminished effect of UV-C light due to roughness and uneven surface of apples.

Based on the outcomes of the aforementioned trials, it was evident that doses of 100, 150, and 300 mJ/cm² resulted in appreciable reductions. While a slight degree of dehydration and shriveling was observed at 300 mJ/cm², the doses of 100 and 150 mJ/cm² were selected for further investigation.

4.1.3 Sanitised and Unsanitised Mesclun

Table 4.3 Microbial log reductions (log CFU/cm^2) of sanitised and unsanitised mesclun after UV-C treatment

Trial	Dosage mJ/cm^2	Unsanitised Mesclun		Sanitised Mesclun		Visual observations
		Initial mean after treatment log CFU/cm^2	Log Reduction log CFU/cm^2	Initial mean after treatment log CFU/cm^2	Log Reduction log CFU/cm^2	
1	**0**	***6.77 ± 0.07**		***6.15 ± 0.03**		Leaves with smooth surface, stable structure and colour
	100	4.52 ± 0.02	2.25	4.87 ± 0.04	1.28	No visible dehydration and similar to the control leaves
	150	4.52 ± 0.06	2.25	5.12 ± 0.06	1.03	No changes in the physical appearance or the texture of the leaves
	200	4.19 ± 0.06	2.58	4.65 ± 0.17	1.50	Fragile and dehydrated leaves with loose structure
	300	3.73 ± 0.03	3.03	5.21 ± 0.05	0.94	Highly dehydrated, sticky and shrivelled
2	**0**	***6.53 ± 0.23**		***6.89 ± 0.10**		Tender, green leaves with no damage
	100	5.22 ± 0.08	1.30	5.04 ± 0.02	1.84	No wilting, discolouration and visible dehydration
	150	5.50 ± 0.03	1.02	5.14 ± 0.12	1.74	No changes in the texture, shape and no visible dehydration

Notes:

- **Bold*** non-UV-C treated control mesclun sample
- n (sample size) = 2;
- Log reduction = (initial mean of control) – (initial mean of dosage)
- (±) Standard deviation of the mean of two samples

The total colony forming units for the unsanitized and sanitized control mesclun samples were 6.77 ± 0.07 and 6.15 ± 0.03 log CFU/cm², respectively. Doses of 100, 150, 200, and 300 mJ/cm² resulted in log reductions ranging from 2.25 to 3.03 log CFU/cm² for unsanitized mesclun. Although all the doses yielded favorable results, doses of 200 and 300 mJ/cm² led to leaf dehydration and shriveling. In the case of sanitized mesclun, a dose of 100 mJ/cm² produced a substantial log reduction of 1.28 CFU/cm², while 200 mJ/cm² also demonstrated an effective reduction of 1.50 log CFU/cm².

In the second trial for mesclun, doses of 100 and 150 mJ/cm² were selected. The total viable counts for the unsanitized control sample were 6.77 ± 0.07 log CFU/cm², and for the sanitized control sample, it was 6.15 ± 0.03 log CFU/cm². A significant log reduction of 1.30 was observed for the 100 mJ/cm² dosage in the case of unsanitized mesclun. Doses of 100 and 150 mJ/cm^2 resulted in microbial reductions of 1.84 and 1.74 log CFU/cm^2, respectively, for the sanitized sample.

Based on the observations, it can be concluded that dosages of 100 and 150 mJ/cm^2 would be suitable for both sanitized and unsanitized mesclun. These doses provide a substantial log reduction without compromising the quality of the leaves.

4.1.4 Sanitised and Unsanitised Rocket

Table 4.4 Microbial log reductions (log CFU/cm^2) of sanitised rocket after UV-C treatment

Trial	**Dosage mJ/cm^2**	**Sanitised Rocket**		**Unsanitised Rocket**		**Visual Observation**
		Initial mean after treatment log CFU/cm^2	**Initial mean after treatment log CFU/cm^2**	**Initial mean after treatment log CFU/cm^2**	**Initial mean after treatment log CFU/cm^2**	
1	**0**	***5.28 ± 0.05**		***5.79 ± 0.04**		Smooth surfaced leaves with firm structure and texture.
	100	4.33 ± 0.10	0.94	4.59 ± 0.03	1.19	Firm and stable leaves with a slight shrivelling
	150	4.33 ± 0.03	0.94	4.7 ± 0.02	1.08	Minimal dehydration was observed, making the leaves soft.
2	**0**	***7.09 ± 0.01**		***5.78 ± 0.02**		Fresh, green leaves with no visible damage
	100	5.03 ± 0.08	2.06	4.25 ± 0.02	1.24	No changes in the physical appearance of the leaves.
	150	5.20 ± 0.05	1.89	4.53 ± 0.02	1.52	No visible shrivelling or dehydration noted
	200	5.02 ± 0.14	2.06	4.61 ± 0.01	1.17	Slight dehydration but firm leaves
3	**0**	***5.37 ± 0.11**		***5.84 ± 0.04**		Fresh leaves with no visible damage and firm structure
	100	3.44 ± 0.13	1.93	4.68 ± 0.03	1.26	Similar to fresh leaves with no visible dehydration
	150	3.82 ± 0.03	1.54	4.57 ± 0.04	1.16	Good texture, no visible dehydration and colour change

Note:

- **Bold* =** non-UV-C treated control rocket sample
- n (sample size) = 2;
- Log reduction = (initial mean of control) – (initial mean of dosage)
- (±) Standard deviation of the mean of two samples

In the first trial, the mean colony forming unit for the controlled sanitized and unsanitized rocket was 5.28 ± 0.05 and 5.79 ± 0.04 log CFU/cm² respectively. No significant log reduction was observed at the dosage of 100 and 150 mJ/cm² for sanitized rockets, whereas for unsanitized rocket, a reduction of 1.19 and 1.08 log CFU/cm² was observed for both 100 and 150 mJ/cm² dosage.

In the second trial of the rocket, UV-C doses ranging from 100-200 mJ/cm² were employed. A substantial log reduction was observed in the sanitized rocket for all three doses. The initial microbial count for the control sample was 7.09 ± 0.01 log CFU/cm². Both doses of 100 and 200 mJ/cm² produced a considerable reduction of 2.06 log CFU/cm² for sanitized rocket. For unsanitized samples, all three doses yielded similar reductions of 1.24, 1.52, and 1.17 log CFU/cm² for 100, 150, and 200 mJ/cm², respectively. Although 200 mJ/cm² achieved appreciable reduction for both sanitized and unsanitized rocket, it also caused minor dehydration of the rocket leaves. As a result, doses of 100 and 150 mJ/cm² were considered for the third trial.

In the third trial of sanitized rocket, both doses of 100 and 150 mJ/cm² demonstrated effective reductions of 3.44 ± 0.13 and 3.82 ± 0.03 log CFU/cm² for the sanitized rocket, and 4.68 ± 0.03 and 4.57 ± 0.04 for the unsanitized rocket. These selected dosages did not compromise the quality of the leaves.

Based on the results obtained in phase I, it can be concluded that UV-C dosages of 100 and 150 mJ/cm² were optimal choices considering the minimum exposure time, maximum microbial reduction, and no observable physical changes to the sanitized and unsanitized leafy salads.

4.2 Phase II- Standardization of UV-C dosage between 100 and 150 mJ/cm^2

In phase II of the experiments, the sanitised and unsanitised spinach, kale, rocket and mesclun were treated with UV-C dosages of 0, 100 and 150 mJ/cm^2. The total plate count of the samples was analysed and the results were used to generate a general full factorial design using Minitab 19 (Minitab Inc. USA) with factors: UV-C dosage, type of salad leaf and sanitization ($\alpha = 0.05$).

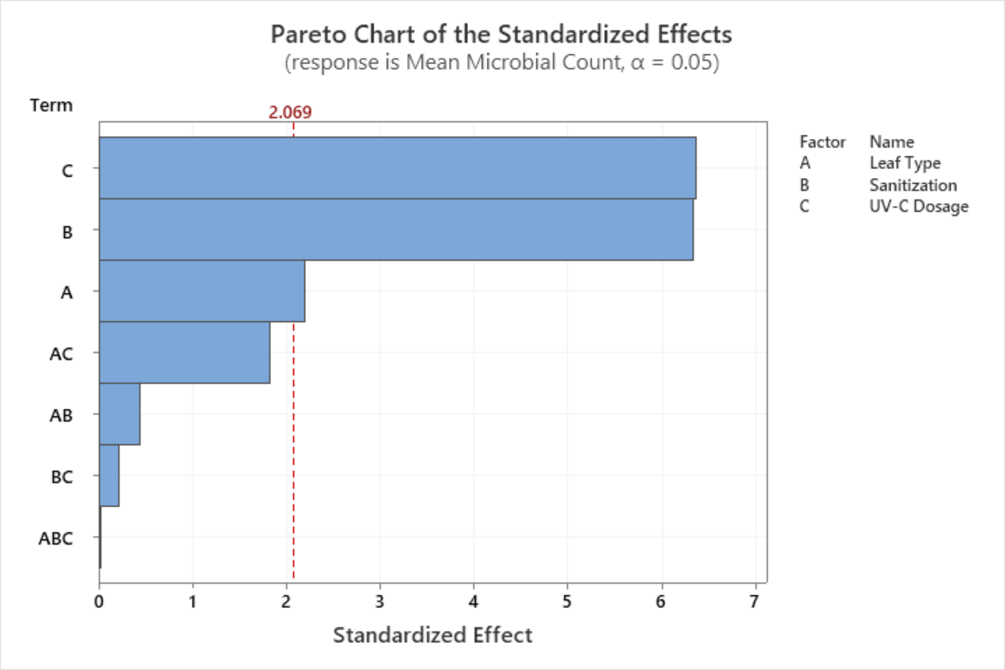


Figure 4.1 Effect of factors (leaf type, sanitization and dosage) on microbial counts.

Note: A: leaf type; B: sanitization; C: UV-C dosage; AB: interaction between leaf type and sanitization; AC: interaction between leaf type and UV-C dosage; BC: interaction between sanitization and UV-C dosage; ABC: interaction between leaf type, sanitization and UV-C dosage

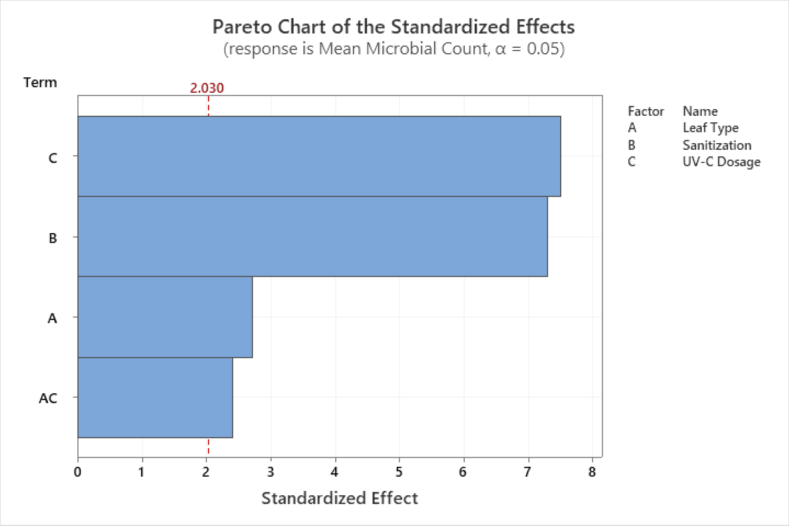


Figure 4.2: Effect of major factors (leaf type, sanitization and dosage) on microbial counts.
Note: A: leaf type; B: sanitization; C: UV-C dosage; AB: interaction between leaf type and sanitization; AC: interaction between leaf type and UV-C dosage.

The Pareto chart of standardised effects was generated as shown in figure 4.1 and 4.2. The factors A (leafy green vegetables), B (sanitization) and C (UV-C dosage), along with A*C (interaction between salad leaf type and UV-C dosage) had major effects on the viable cell counts.

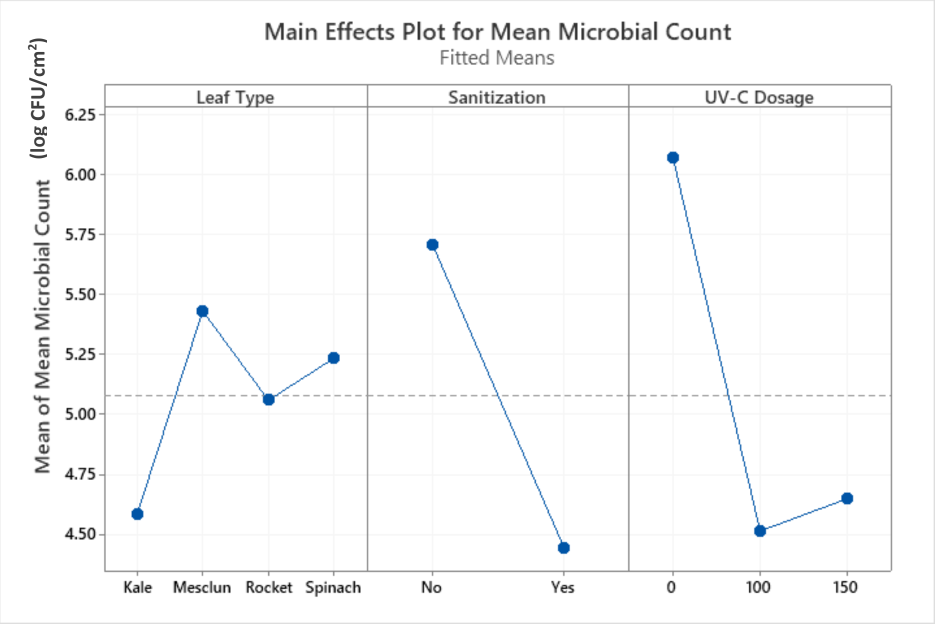


Figure 4.3: The main effects plot on significant main effects
Note: The graph was generated by minitab by using the mean of mean microbial counts

The main effect plot in Figure 4.3 represents the effect of each factor (salad leaf type, sanitization and UV-C dosage) on the mean microbial count. Kale had the least mean microbial count whereas mesclun had the highest mean microbial count. The sanitised salads had significantly lower ($\alpha = 0.05$) number of microbial counts compared to the unsanitised salads. The non-UV-C treated salads had high microbial cell counts of 6.06 log CFU/cm^2. The UV-C dosage of 150 mJ/cm^2 had slightly higher mean viable cell counts of 4.65 log CFU/cm^2 compared to 100 mJ/cm^2 with viable cell counts of 4.51 log CFU/cm^2.

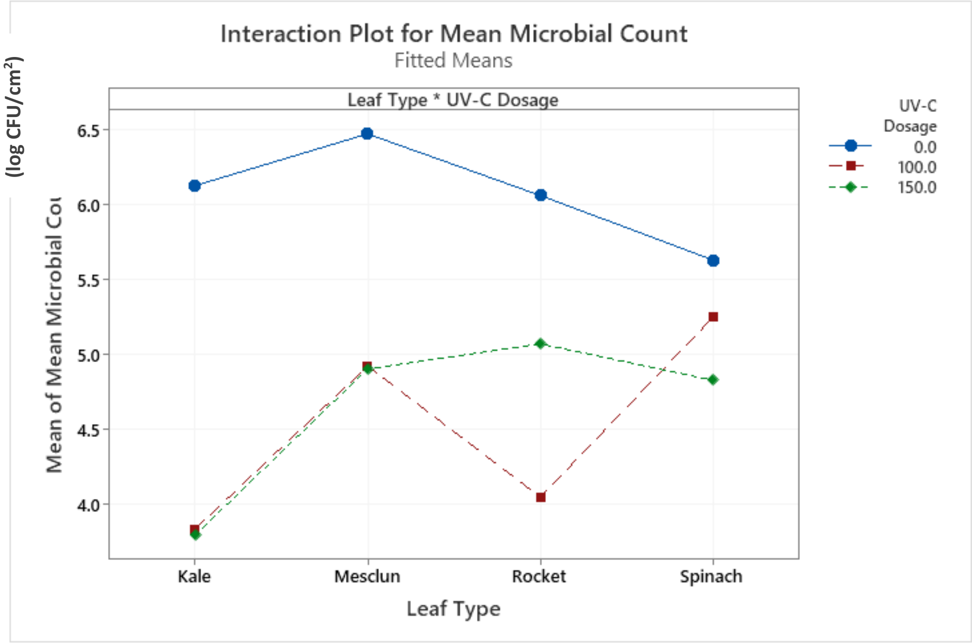


Figure 4.4 interaction plot showing the significant two-way interaction between Leaf Type*UV-C Dosage
Note: The graph was generated by minitab by using the mean of mean mcrobial count

The interaction plot in Figure. 4.4 shows the interaction between the salad leaf type and the UV-C dosage. It can be observed that kale had the highest effect of UV-C treatment on the mean microbial count and spinach has the least effect. The residual plot (Appendix A4) fulfilled the assumptions of normality, equal variance and independence and proved that the factors were a good fit for the data. A good outcome of adjusted R^2 of 74.27 % (Appendix 2) of the final model showed that most of the variability of the viable cell counts observed was explained by the factors used. Hence it was an appropriate model.

The results in Phase II suggest that the salad leafy green type, sanitisation and the UV-C dosage had major effects on the mean viable cell counts. Mesclun had higher microbial counts followed by spinach, rocket and kale. Based on the results, the sanitised salads treated with UV-C dosage of 100 mJ/cm^2 had the lowest number of mean viable cell counts. Hence, the UV-C dosage of 100 mJ/cm^2 was selected for further work in phase III.

4.3 PHASE III – Shelf-life study of standard UV-C (100mJ/cm^2) treated and untreated RTE leafy salads

4.3.1 Microbial Analysis

I) Spinach (Day 0 – 12)

Table 4.5: Microbial count of UV-C treated and untreated sanitised and unsanitised kale during storage at 4° C for 12 days

Storage time (day)	**Dosage (mJ/cm^2)**	**Unsanitised Spinach**		**Sanitised Spinach**	
		Initial mean after treatment (log CFU/cm^2)	**Reduction (log CFU/cm^2)**	**Initial mean after treatment (log CFU/cm^2)**	**Log Reduction (log CFU/cm^2)**
0	0	5.36 ± 0.0	1.43	4.94 ± 0.08	1.35
	100	3.9 ± 0.02		3.58 ± 0.11	
4	0	7.61 ± 0.03	3.05	7.06 ± 0.05	2.60
	100	4.55 ± 0.04		4.45 ± 0.01	
8	0	8.91 ± 0.03	1.03	8.42 ± 0.0	1.69
	100	7.88 ± 0.02		6.73 ± 0.07	
12	0	9.35 ± 0.04	1.27	9.24 ± 0.07	1.39
	100	8.08 ± 0.09		7.84 ± 0.01	

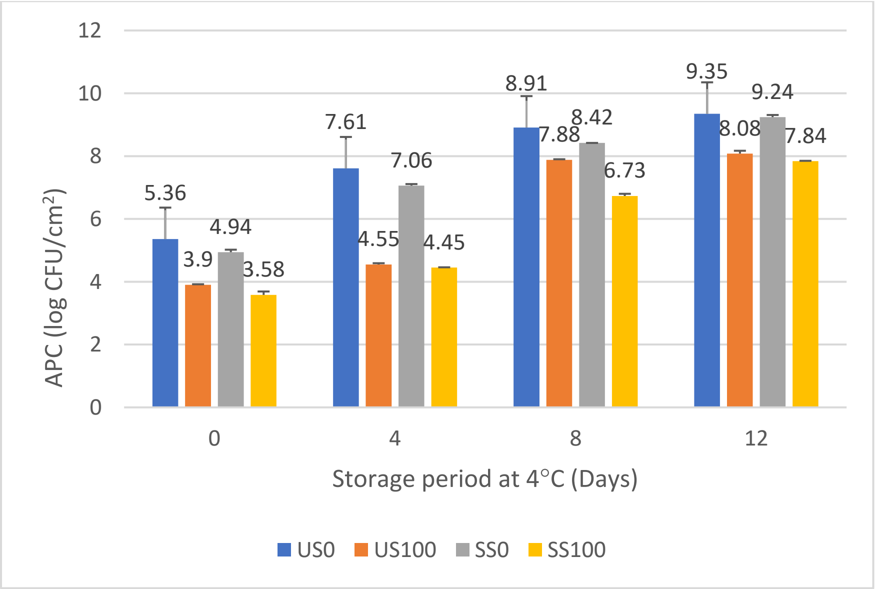


Figure 4.5 Microbial count (log CFU/cm^2) of UV-C treated and untreated sanitised and unsanitised spinach during storage at 4° C for 12 days

Note: US0: Unsanitised spinach control; US100: Unsanitised spinach treated with 100 mJ/cm^2; SS0: Sanitised spinach control; SS100: Sanitised spinach treated with 100 mJ/cm^2

Over the storage period of 12 days at 4° C the microbial count of US0 increased from 5.36 log CFU/cm2 to 9.35 log CFU/cm^2. SS100 sample had the least increase in the microbial count from 3.58 log CFU/cm^2 to 7.84 log CFU/cm^2 over the storage period. The initial and final microbial count for US100 was 3.9 log CFU/cm^2 and 8.08 log CFU/cm^2 respectively. 3.05 log CFU/cm^2 was the highest log reduction for unsanitised spinach on day 4 of storage whereas 2.60 log CFU/cm^2 is the highest log reduction for sanitised spinach on day 4. From figure 4.5 it can be observed that SS100 is the best as it has the lowest number of microbial count on the last day of shelf life.

Nguyen-The and Carlin (1994) have reported similar microbial counts in minimally processed vegetables, ranging from 3 to 6 log CFU/cm^2 just after processing and from 3 to 9 log CFU/cm^2 after commercial cold storage. Baby spinach leaves treated with high doses of UV-C (12 or 24 kJ/m^2) had reductions of between 1 or 1.5 log CFU/cm^2 with respect to the control samples (Escalona et al., 2010).

Enumeration of *L. monocytogenes,* coliforms, *S. aureus* and *Salmonella* spp was conducted for all the four spinach samples, US0, US100, SS0 and SS100. None of the pathogens were detected in any of the samples during the 12 days of storage at 4 °C.

From Appemdix B2, it can be observed that all the 3 factors, product, storage time and dosage had a significant effect ($p<0.05$) on the total microbial cell count of the spinach samples during the storage period of 12 days at 4°C. Appendix B3 also shows that the mean microbial cell count was higher for unsanitised spinach as compared to sanitised spinach. Over the storage period of 0, 4, 8 and 12 days, the mean microbial count increased. A significant difference in the mean microbial count was observed between 100 mJ/cm^2 dosage and the control 0 mJ/cm^2.

II) Kale (Day 0 – 12)

Table 4.6: Microbial count of UV-C treated and untreated sanitised and unsanitised kale during storage at 4° C for 12 days

Day	Dosage mJ/cm^2	Unsanitised Kale		Sanitised Kale	
		Initial mean after treatment log CFU/cm^2	Log Reduction log CFU/cm^2	Initial mean after treatment log CFU/cm^2	Log Reduction log CFU/cm^2
0	0	6.44 ± 0.09	2.93	4.54 ± 0.00	1.47
	100	3.51 ± 0.04		3.07 ± 0.03	
4	0	6.67 ± 0.01	1.02	5.41 ± 0.04	1.39
	100	5.64 ± 0.03		4.01 ± 0.05	
8	0	7.22 ± 0.04	1.19	6.64 ± 0.07	1.73
	100	6.03 ± 0.03		4.90 ± 0.02	
12	0	7.78 ± 0.07	1.18	6.74 ± 0.04	1.30
	100	6.59 ± 0.09		5.44 ± 0.04	

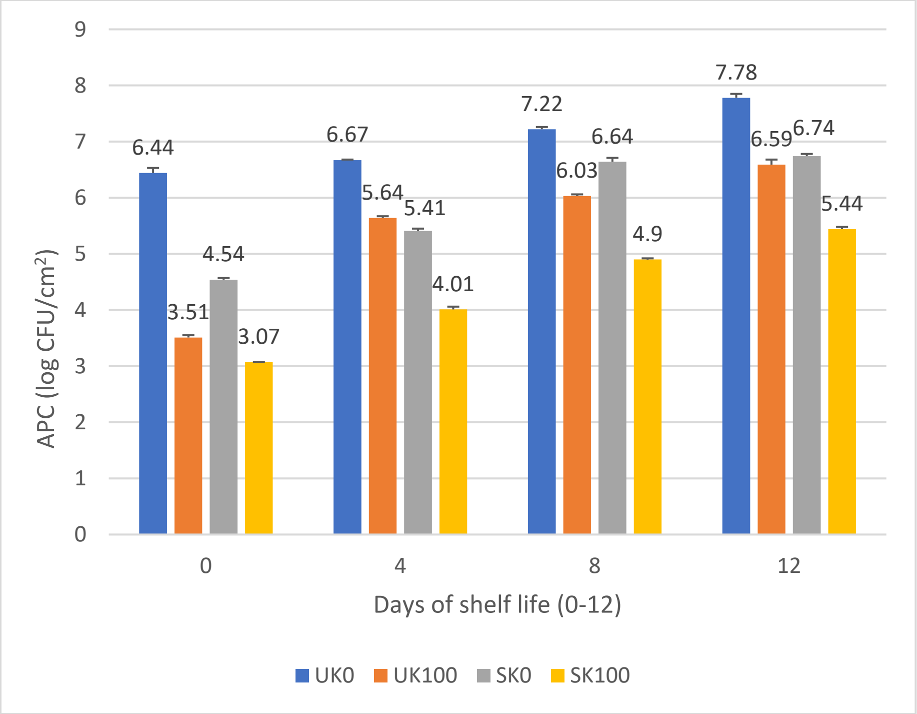


Figure 4.6 Microbial count (log CFU/cm^2) of UV-C treated and untreated sanitised and unsanitised kale during storage at 4° C for 12 days

Note: UK0: Unsanitised kale control; UK100: Unsanitised kale treated with 100 mJ/cm^2; SK0: Sanitised kale control; SK100: Sanitised kale treated with 100 mJ/cm^2

During the 12-day shelf life, the microbial count of UK0 increased the most, rising from 6.44 log CFU/cm² to 7.78 log CFU/cm². On the other hand, the SK100 sample exhibited the smallest increase in microbial count, ranging from 3.07 log CFU/cm² to 5.44 log CFU/cm² throughout the storage period. For the US100 sample, the initial and final microbial counts were 3.9 log CFU/cm² and 8.08 log CFU/cm² respectively.

UK100 experienced an increase in microbial count from 3.5 log CFU/cm² to 6.59 log CFU/cm², while SK0 showed an increase from 4.54 log CFU/cm² to 6.74 log CFU/cm². Based on the graph, it can be concluded that SK100 performed the best, as it exhibited the least growth of microbial colonies over the 12-day storage period at 4°C.

Enumeration of *L. monocytogenes, coliforms. S. aureus* and *Salmonella* spp was conducted for all the four kale samples, UK0, UK100, SK0 and SK100. None of the pathogens were detected in any of the samples during the 12 days of storage at 4 °C.

All the three factors, product, storage time and dosage had a significant effect on the microbial cell count of kale ($p<0.05$). The difference between the mean microbial cell count of sanitised and unsanitised kale were significant ($p<0.05$). Significant differences ($p<0.05$) were also observed in the microbial count on day 0, 4, 8 and 12. Substantial changes ($p<0.05$) in the microbial count were observed between the control and 100 mJ/cm^2 dosage.

Appendix B6 illustrates that, sanitised kale had lower mean microbial cell count in contrast to unsanitised kale. The mean microbial cell count increased significantly ($p<0.05$) over the period of 12 days. Dosage 100 mJ/cm^2 had significantly lower microbial count than 0 mJ/cm^2

III) Mesclun (Day 0 – 12)

Table 4.7 Microbial count of UV-C treated and untreated sanitised and unsanitised mesclun during storage at 4° C for 12 days

Day	**Dosage mJ/cm^2**	**Unsanitised Mesclun**		**Sanitised Mesclun**	
		Initial mean after treatment log CFU/cm^2	**Log Reduction log CFU/cm^2**	**Initial mean after treatment log CFU/cm^2**	**Log Reduction log CFU/cm^2**
0	0	7.26 ± 0.23	1.56	7.02 ± 0.08	1.47
	100	5.7 ± 0.00		5.55 ± 0.01	
4	0	8.00 ± 0.00	1.09	7.66 ± 0.04	1.08
	100	6.91 ± 0.01		6.57 ± 0.05	
8	0	9.15 ± 0.07	1.35	9.01 ± 0.01	1.42
	100	7.79 ± 0.02		7.59 ± 0.07	
12	0	9.17 ± 0.02	1.41	9.05 ± 0.03	1.59

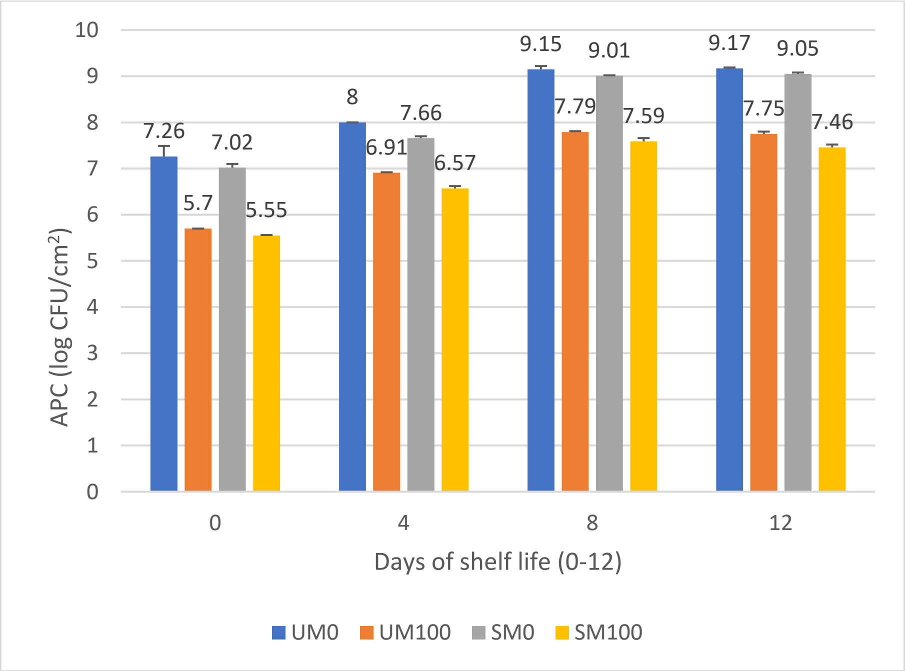


Fig 4.7 Microbial count (log CFU/cm^2) of UV-C treated and untreated sanitised and unsanitised mesclun during storage at 4° C for 12 days

Note: UM0: Unsanitised mesclun control; UM100: Unsanitised mesclun treated with 100 mJ/cm^2; SM0: Sanitised mesclun control; SM100: Sanitised mesclun treated with 100 mJ/cm^2

The Figure 4.7 above depicts the microbial counts for both sanitized and unsanitized mesclun on days 0, 4, 8, and 12 of storage. Observing the figure, it becomes evident that the microbial count over the 12-day period of UM0 experienced the most substantial increase, ranging from 7.26 log CFU/cm² to 9.17 log CFU/cm². In contrast, SM100 displayed the least growth in microbial count, escalating from 5.55 log CFU/cm² to 7.46 log CFU/cm² throughout the storage period. SM0 exhibited an increase in microbial count, ranging from 7.02 log CFU/cm² to 9.05 log CFU/cm², whereas UM100 displayed an increase from 5.7 log CFU/cm² to 7.75 log CFU/cm².

Enumeration of *L. monocytogenes,* coliforms, *S. aureus* and *Salmonella* spp was conducted for all the four mesclun samples, UM0, UM100, SM0 and SM100. None of the pathogens were detected in any of the samples during the 12 days of storage at 4 °C.

The mean microbial cell count of mesclun is significantly affected by all the three factors, product type, storage time and dosage ($p<0.05$). The microbial count was less in sanitised mesclun in comparison to unsanitised mesclun., and the difference in the microbial cell count between sanitised and unsanitised mesclun was significant ($p<0.05$). The mean microbial count during the storage time increased significantly ($p<0.05$) from day 0 to day 8 but, during day 12 the microbial count decreased as shown in figure 4.7. The mean microbial count for mesclun samples treated with UV-C dosage 100 mJ/cm^2 had significantly lower microbial count compared to the control samples.

IV) Rocket (Day 0 – 12)

Table 4.8 Microbial count of UV-C treated and untreated sanitised and unsanitised rocket during storage at 4° C for 12 days

Day	Dosage mJ/cm^2	Unsanitised Rocket		Sanitised Rocket	
		Initial mean after treatment log CFU/cm^2	**Log Reduction log CFU/cm^2**	**Initial mean after treatment log CFU/cm^2**	**Log Reduction log CFU/cm^2**
0	0	6.62 ± 0.02	1.98	5.52 ± 0.1	1.88
	100	4.64 ± 0.07		3.64 ± 0.11	
4	0	8.16 ± 0.00	1.05	7.1 ± 0.02	1.15
	100	7.1 ± 0.03		6.04 ± 0.00	
8	0	8.73 ± 0.03	1.1	7.97 ± 0.00	1.09
	100	7.63 ± 0.02		6.88 ± 0.03	
12	0	9.37 ± 0.00	0.89	9.06 ± 0.02	1.24
	100	8.47 ± 0.09		7.81 ± 0.03	

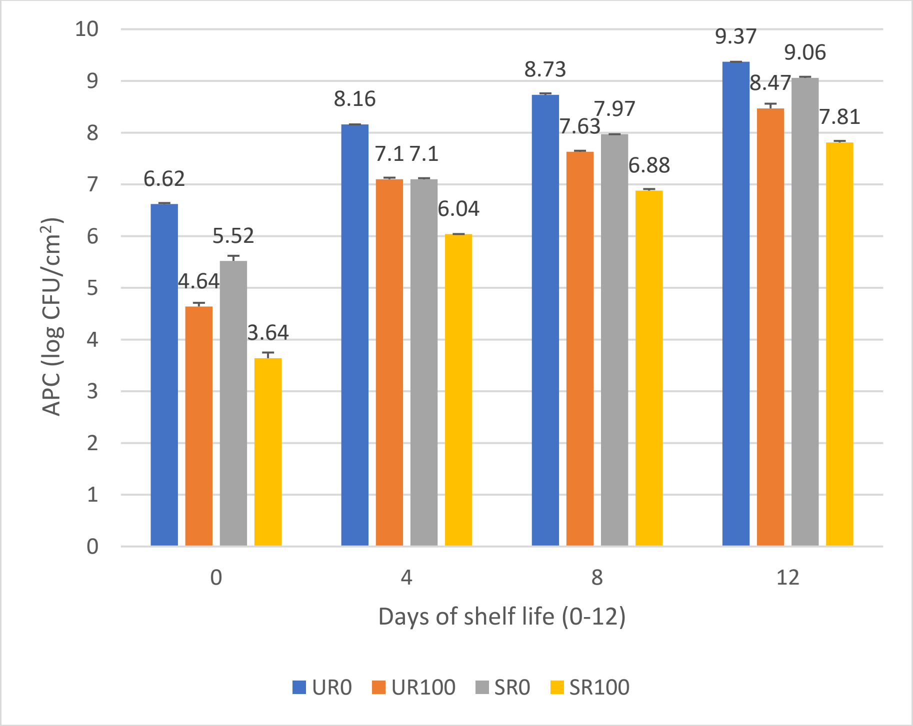


Figure 4.8 Microbial count (log CFU/cm^2) of UV-C treated and untreated sanitised and unsanitised rocket during storage at 4° C for 12 days

Note: UR0: Unsanitised rocket control; UR100: Unsanitised rocket treated with 100 mJ/cm^2; SR0: Sanitised rocket control; SR100: Sanitised rocket treated with 100 mJ/cm^2

The Figure 4.8 above illustrates the microbial counts for both sanitized and unsanitized rocket on days 0, 4, 8, and 12 of storage. It is evident that the microbial count for UR0 experienced a substantial increase, escalating from 6.62 log CFU/cm^2 to 9.37 log CFU/cm^2. In contrast, SR100 displayed the smallest growth in microbial count, going from 3.64 log CFU/cm^2 to 7.81 log CFU/cm^2 during the storage period. SR0 showed an increase in microbial count from 5.52 log CFU/cm^2 to 9.06 log CFU/cm^2, while UR100 exhibited an increase from 4.64 log CFU/cm^2 to 8.47 log CFU/cm^2.

A similar result was obtained in a previous study where different UV-C dosages (10, 20, and 30 kJ/m^2) were found to be effective in inhibiting the growth of natural microflora on fresh rocket leaves during storage. However, contrary to the present results, no significant differences among treatments or with the control were observed on day 12 (Gutiérrez et al., 2015).

Enumeration of *L. monocytogenes,* coliforms, *S. aureus* and *Salmonella* spp was conducted for all the four kale samples, UR0, UR100, SR0 and SR100. None of the pathogens were detected in any of the samples during the 12 days of storage at 4 °C.

All the three factors A, B and C which represents the product, storage time and dosage respectively, had significant effects on the microbial cell count of rocket ($p<0.05$) as shown in Appendix B11. The main effect plot in Appendix B12 shows that the difference in the microbial counts within the products, storage time and dosage were significant ($p<0.05$).

The microbial count for unsanitised rocket was greater than the sanitised rocket. The mean microbial count of rocket vastly increased from day 0 to 4. On day 4 to 12 the microbial count increased gradually. The microbial count for the control dosage 0 mJ/cm^2 was significantly higher than for dosage 100 mJ/cm^2.

4.3.2 Colour Analysis

I) Spinach

- **Lightness**

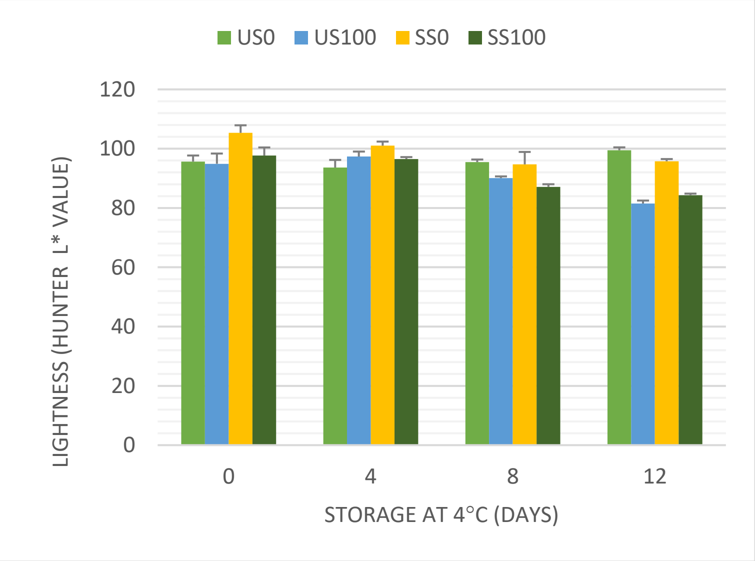


Figure 4.9 Changes in the Hunter L* (lightness) value of UV-C treated and untreated sanitised and unsanitised spinach during storage at 4° C for 12 days

Note: US0: Unsanitised spinach control; US100: Unsanitised spinach treated with 100 mJ/cm^2; SS0: Sanitised spinach control; SS100: Sanitised spinach treated with 100 mJ/cm^2

From Figure 4.9, it's apparent that there is a slight increase in lightness for US0, which rose from 95.65 to 99.46 over the 12-day storage period. The lightness of US100, however, decreased from 94.91 to 81.51 between day 0 and day 12.

Across the span of 12 days, both SS0 and SS100 experienced reductions in lightness, transitioning from 105.33 to 95.76 and from 97.71 to 84.34, respectively. This observation aligns with the findings of Artés-Hernández et al. (2009), where spinach samples treated with UV-C doses displayed a significant decrease in L* values by the end of the storage period, declining by up to 10–15% of the initial level. This decrease is likely attributed to cellular damage caused by the UV-C doses. A similar tendency towards reduced L* values with UV-C treatments was noted by Martínez-Sánchez et al. (2019), although these changes were not statistically significant.

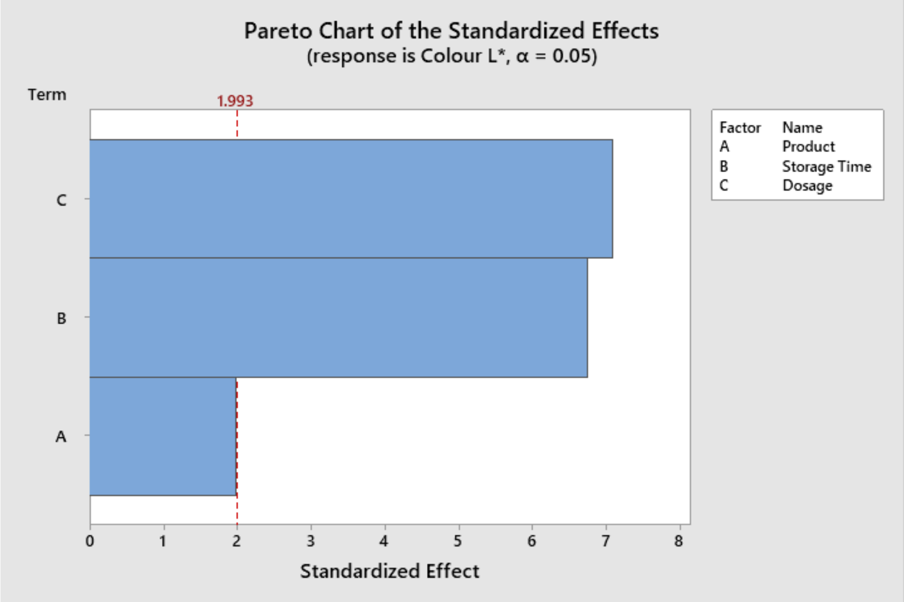


Figure 4.10 Pareto Chart of standardised effects for L* (lightness) of spinach
Note: A: Product; B: Storage time; C: UV-C dosage

From the Pareto chart in figure 4.10, it can be observed that the storage time and the dosage had significant effect ($p<0.05$) on the lightness L* of spinach. From the results obtained from ANOVA it was observed that the difference in the lightness between sanitised and unsanitised spinach is not significant ($p>0.05$).

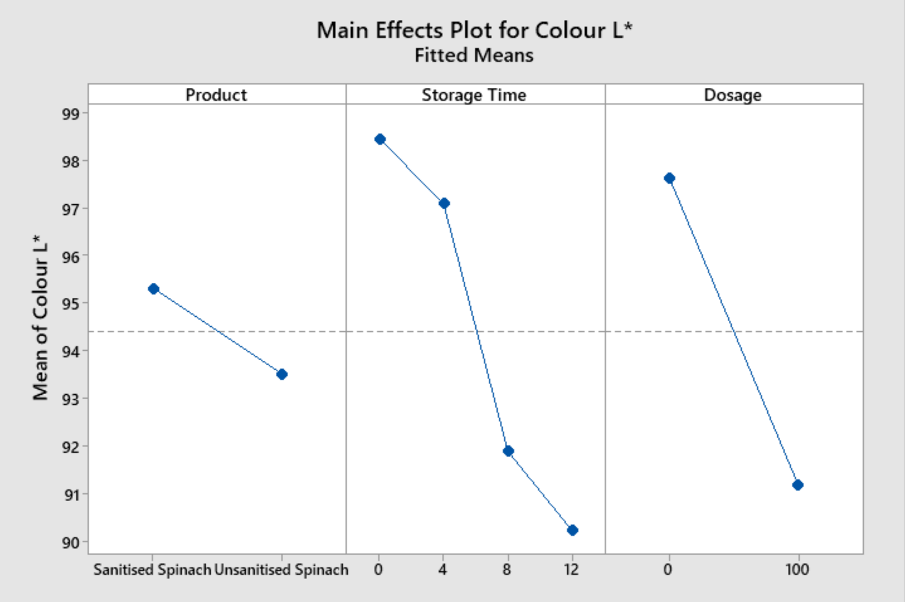


Figure 4.11 Main effect plot for L* (lightness) of spinach
Note: A: Product; B: Storage time; C: UV-C dosage

The figure 4.11 represents the main effect of different factors on the Hunter's L* value. The lightness is higher for the sanitised spinach and lower for unsanitised spinach. The mean lightness (L*) decreased over the 12 days storage period and a drastic fall in the lightness was observed from day 4 to 8. Overall lightness for 0 mJ/cm^2 is higher compared to 100 mJ/cm^2.

- **Greenness**

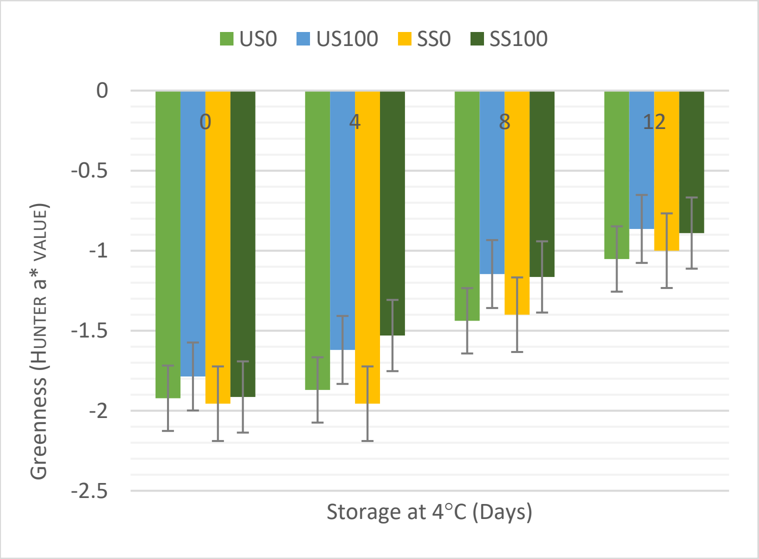


Figure 4.12 Changes in the Hunter a* (greenness) value of UV-C treated and untreated sanitised and unsanitised spinach during storage at 4° C for 12 days
Note: US0: Unsanitised spinach control; US100: Unsanitised spinach treated with 100 mJ/cm^2; SS0: Sanitised spinach control; SS100: Sanitised spinach treated with 100 mJ/cm^2

The greenness of all four samples decreased over the period of 12 days. The greenness of US100 was highly decreased from -1.78 to -0.86. The greenness for US0, SS0 and SS100 was decreased from -1.92 to -1.05, -1.95 to -1.00 and from -1.91 to -0.89 respectively. The green colour is an important quality attribute in spinach leaves which is linked to their freshness, while degreening and yellowing is associated to spinach senescence (Martínez-Sánchez et al., 2019).

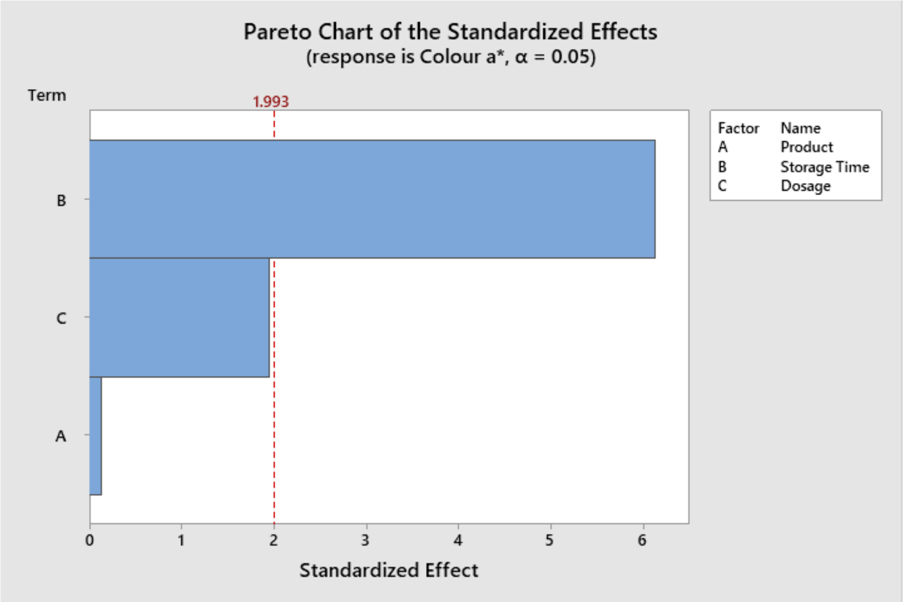


Figure 4.13 Pareto Chart of standardised effects for a* (greenness) of spinach
Note: A: Product; B: Storage time; C: UV-C dosage

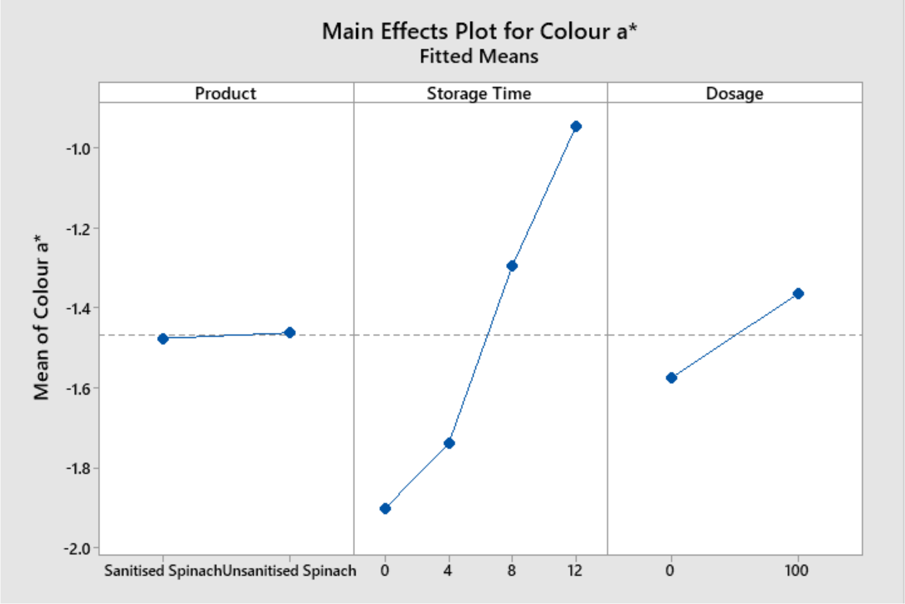


Figure 4.14 Main effect plot for a* (greenness) of spinach
Note: A: Product; B: Storage time; C: UV-C dosage

The storage time is the only factor that has a significant effect ($p<0.05$) on the Hunter's a* value. Factor A and C which represents product and dosage respectively, does not have a significant effect ($p>0.05$) on the greenness (a*).

The main effect plot in figure 4.14 shows that no significant difference (p>0.05) was observed between the products, sanitised spinach and unsanitised spinach and between the dosages, 0 and 100 mJ/cm^2 whereas, significant difference (p<0.05) was observed on various days of the storage.

- **Yellowness**

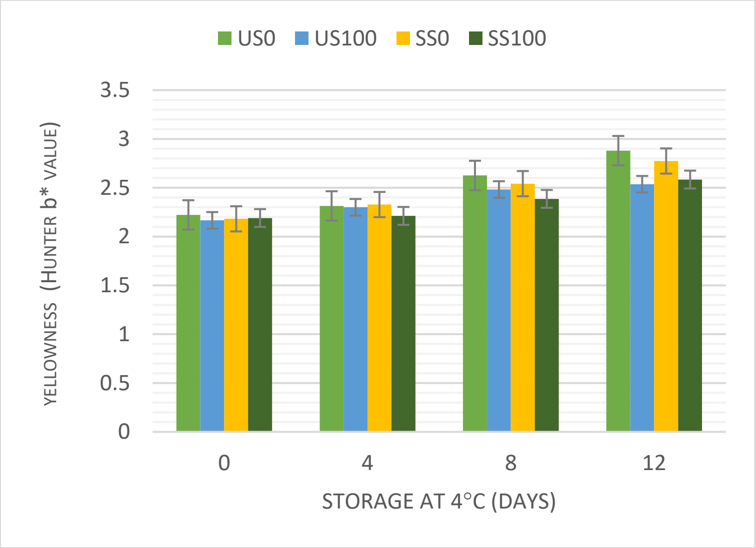


Figure 4.15 Changes in the Hunter b* (yellowness) value of UV-C treated and untreated sanitised and unsanitised spinach during storage at 4° C for 12 days

Note: US0: Unsanitised spinach control; US100: Unsanitised spinach treated with 100 mJ/cm^2; SS0: Sanitised spinach control; SS100: Sanitised spinach treated with 100 mJ/cm^2

The yellowness of all the spinach samples increased during the shelf life of 12 days. For the unsanitised US0 and US100, the yellowness increased slightly from 2.22 to 2.88 and 2.16 to 2.53 respectively. In case of sanitised spinach samples, SS0 and SS100 had increase in the yellowness from 2.18 to 2.77 and 2.19 to 2.58 respectively.

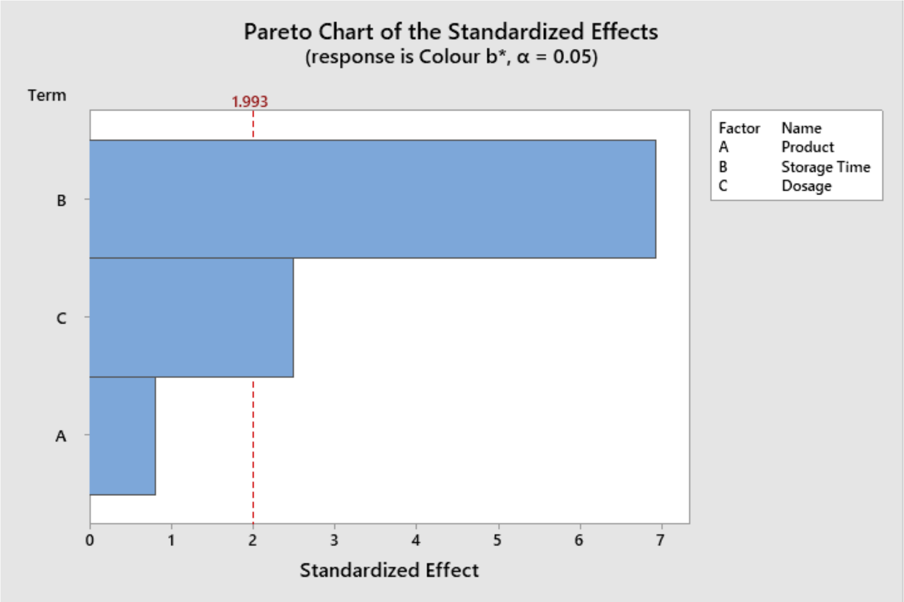


Figure 4.16 Pareto Chart of standardised effects for b* (yellowness) of spinach
Note: A: Product; B: Storage time; C: UV-C dosage

The yellowness (b*) of the spinach sample is majorly affected by two factors, storage time and dosage. Both these factors have a significant effect ($p<0.05$) on the Hunter's b* value whereas product type does not have a significant effect ($p>0.05$) on the yellowness.

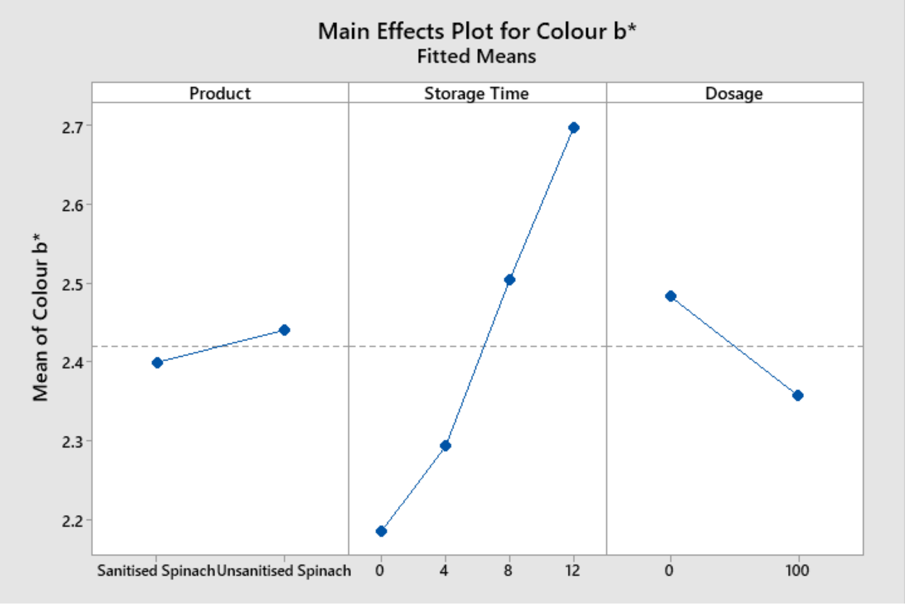


Figure 4.17 Main effect plot for b* (yellowness) of spinach
Note: A: Product; B: Storage time; C: UV-C dosage

From the figure 4.17 it can be observed that, there is no significant difference in yellowness (b*) between sanitised and unsanitised spinach. In case of storage time and dosage, both have significant difference ($p > 0.05$) in yellowness between day 0, 4, 8 and 12 and 0 and 100 mJ/cm^2 respectively.

II) Kale

- **Lightness**

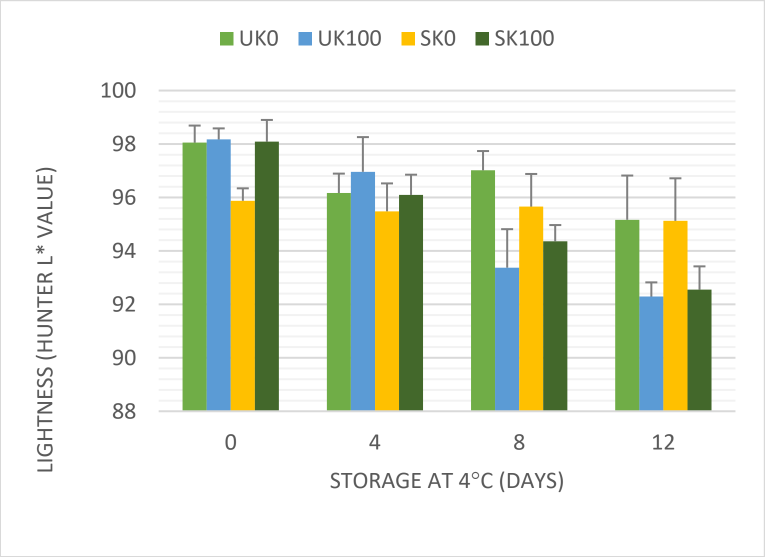


Fig 4.18 Changes in the Hunter L* (lightness) value of UV-C treated and untreated sanitised and unsanitised kale during storage at 4° C for 12 days

Note: UK0: Unsanitised kale control; UK100: Unsanitised kale treated with 100 mJ/cm^2; SK0: Sanitised kale control; SK100: Sanitised kale treated with 100 mJ/cm^2

During the storage period of 12 days at 4° C, the lightness of all the four kale samples decreased. UK0 initially had 98.05 lightness on day 0 which decreased to 96.16 on day 4 and increased to 97.01 on day 8 and decreased again to 95.16.

Both the UV treated UK100 and SK100 had gradual decrease in the lightness from 98.17 to 92.29 and 98.08 to 92.55 respectively. SK0 had gradual decrease in the lightness from 95.87 to 95.48 on day 0 to 4, but a slight increase of 95.65 in the lightness was observed on day 8 and on day 12, the lightness was decreased to 95.12.

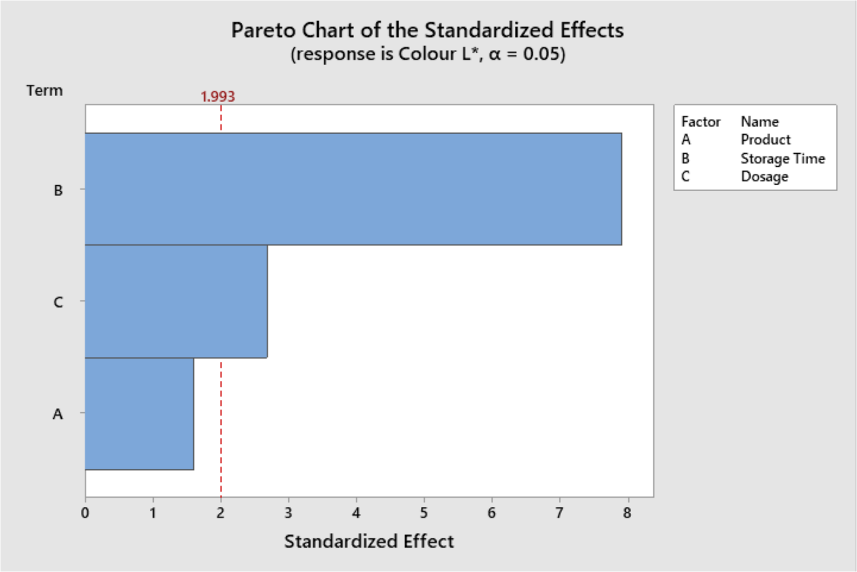


Figure 4.19 Pareto Chart of standardised effects for L* (lightness) of kale
Note: A: Product; B: Storage time; C: UV-C dosage

The Pareto chart in figure 4.19 represents that the factors, storage time and dosage had major effect ($p<0.05$) on the lightness (L*) of kale. The difference between the lightness over various storage time is highly significant ($p<0.05$) and significant differences ($p<0.05$) can also be observed between the different dosages. The difference between the lightness of the sanitised and unsanitised kale products is not significant ($p>0.05$).

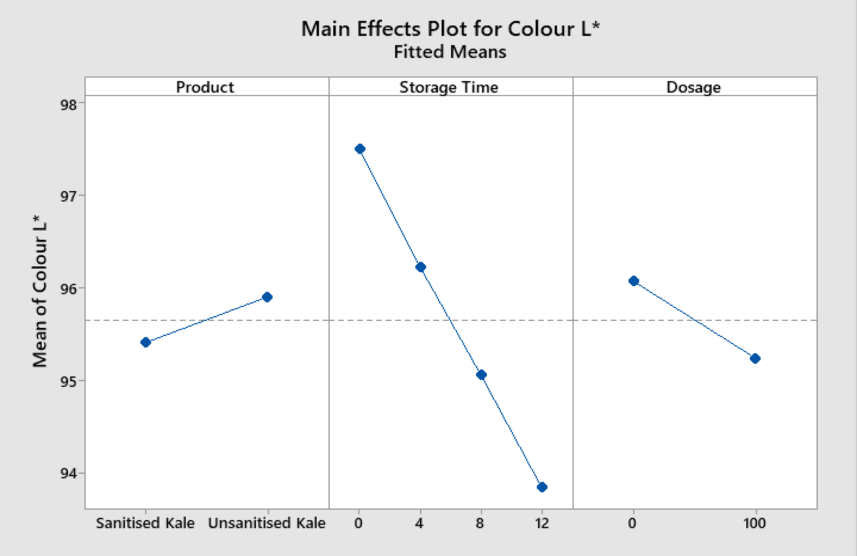


Figure 4.20 Main effect plot for L* (lightness) of kale
Note: A: Product; B: Storage time; C: UV-C dosage

The lightness L* for kale was higher for unsanitised kale and 0 mJ/cm^2 whereas, for sanitised kale and 100 mJ/cm^2 dosage, the mean L* was lower. The mean L* had a gradual decrease from day 0 to day 12 ($p<0.05$).

- **Greenness**

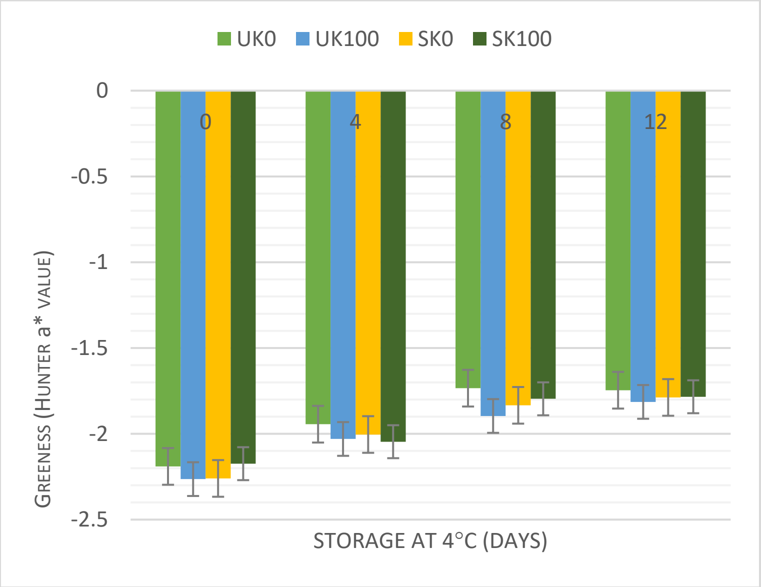


Figure 4.21 Changes in the Hunter a* (greenness) value of UV-C treated and untreated sanitised and unsanitised kale during storage at 4° C for 12 days

Note: UK0: Unsanitised kale control; UK100: Unsanitised kale treated with 100 mJ/cm^2; SK0: Sanitised kale control; SK100: Sanitised kale treated with 100 mJ/cm^2

The greenness of all the four samples decreased over the period of 12 days. For the unsanitised samples UK0 and UK100, the greenness decreased from -2.19 to -1.73 and -2.26 to -1.81 respectively. For sanitised kale samples SK0 and SK100, greenness was reduced from -2.26 to -1.78 and from -2.17 to -1.70 respectively. Highest loss in the greenness was observed for UK0 samples.

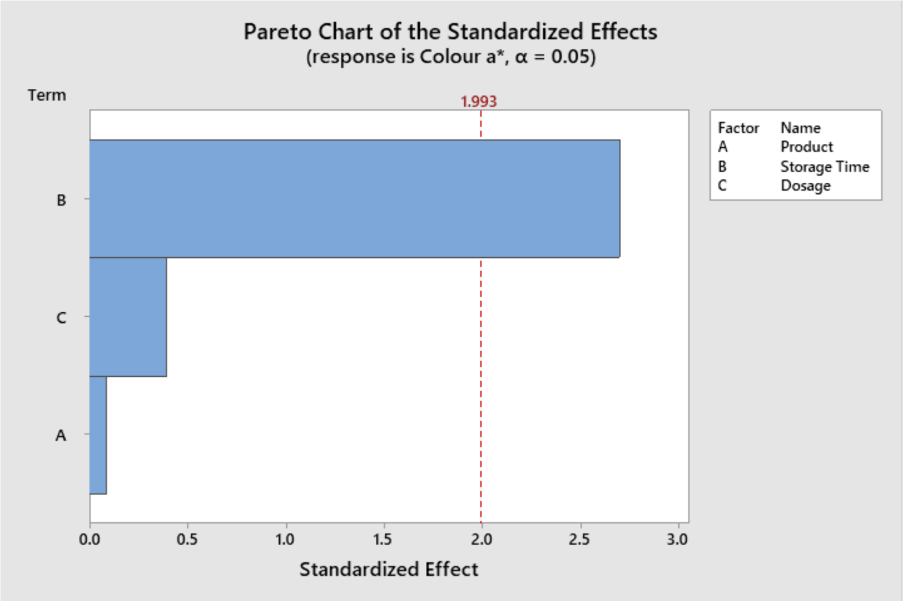


Figure 4.22 Pareto Chart of standardised effects for a* (greenness) of spinach
Note: A: Product; B: Storage time; C: UV-C dosage

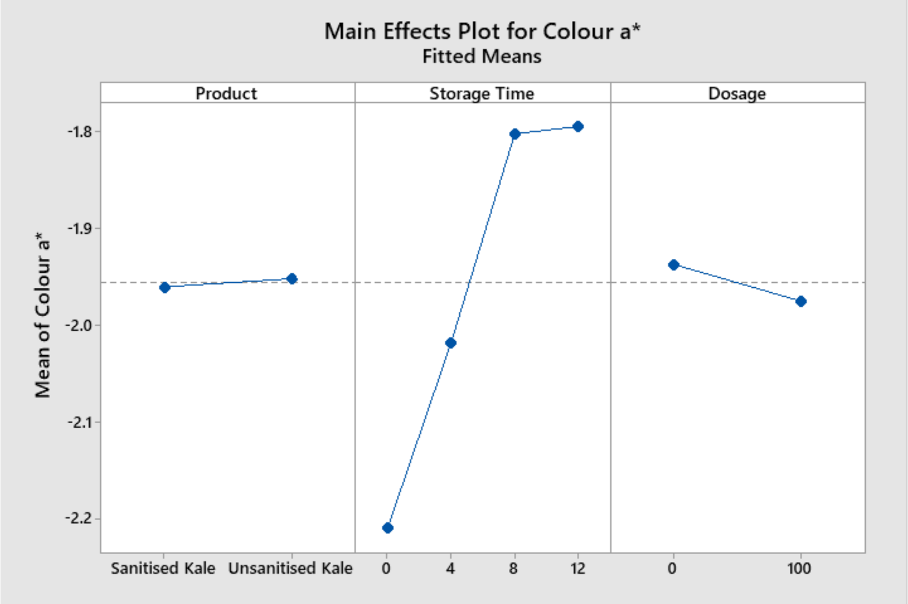


Figure 4.23 Main effect plot for a* (greenness) of kale
Note: A: Product; B: Storage time; C: UV-C dosage

From figure 4.22 and 4.23, it can be observed that out of all the three factors, storage time had the most significant effect on the greenness (a*) ($p<0.05$). The factors product and dosage did not affect the greenness ($p>0.05$).

No significant differences were observed in the lightness of sanitised and unsanitised kale and between the dosages 0 and 100 mJ/cm^2. Significant differences ($p<0.05$) were observed between different days of storage time.

- **Yellowness**

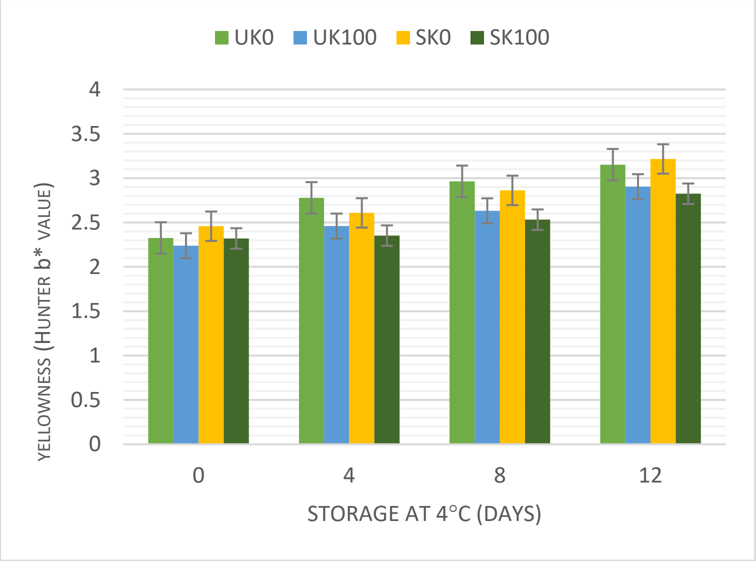


Fig 4.24 Changes in the Hunter b* (yellowness) value of UV-C treated and untreated sanitised and unsanitised spinach during storage at 4° C for 12 days

Note: UK0: Unsanitised kale control; UK100: Unsanitised kale treated with 100 mJ/cm^2; SK0: Sanitised kale control; SK100: Sanitised kale treated with 100 mJ/cm^2

The yellowness in all the four samples was increased during the storage at 4° C for 12 days. SK0 had the highest increase in the yellowness from 2.45 to 3.21 and SK100 had the least increase in the yellowness from 2.32 to 2.82.

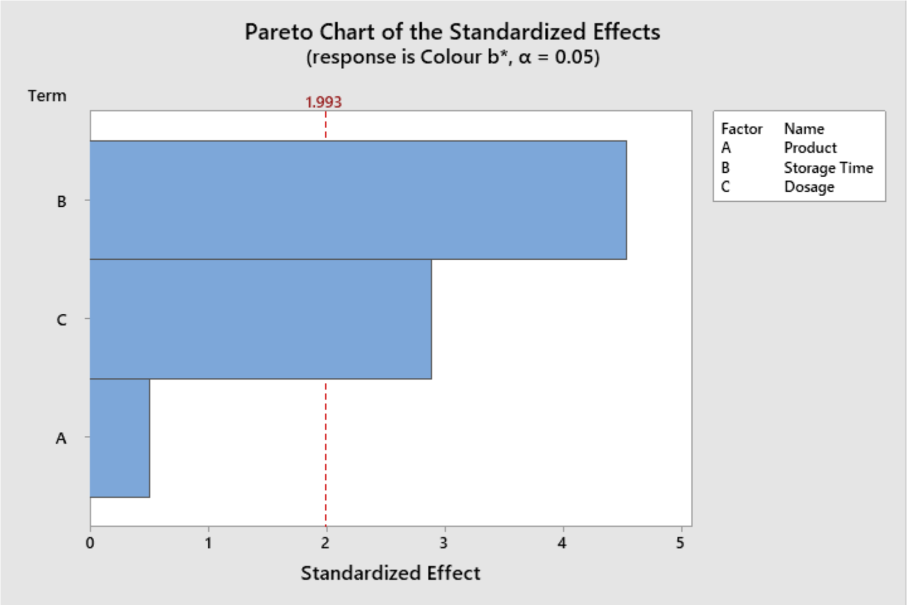


Figure 4.25 Pareto Chart of standardised effects for b* (yellowness) of kale
Note: A: Product; B: Storage time; C: UV-C dosage

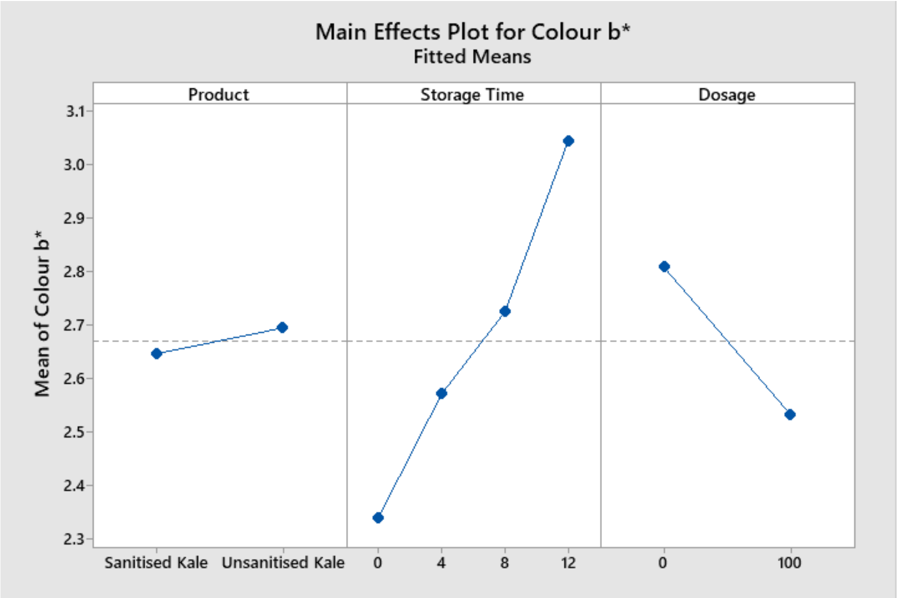


Figure 4.26 Main effect plot for b* yellowness of kale
Note: A: Product; B: Storage time; C: UV-C dosage

From figure 4.25 and 4.26, it was observed that the storage time and dosage majorly affected the yellowness (b*) of kale samples. Sanitised and unsanitised kale did not have a substantial effect ($p>0.05$).

Significant increase in b* value was observed continuously from day 0 to day 12. The mean b* value was high for control sample and low for samples treated with UV-C dosage of 100 mJ/cm^2.

III) Mesclun

- **Lightness**

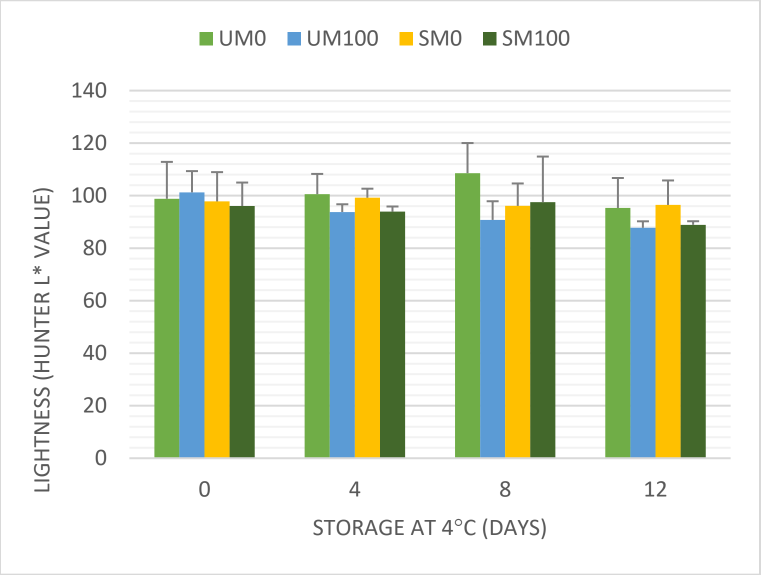


Fig 4.27 Changes in the Hunter L* (lightness) value of UV-C treated and untreated sanitised and unsanitised mesclun during storage at 4° C for 12 days

Note: UM0: Unsanitised mesclun control; UM100: Unsanitised mesclun treated with 100 mJ/cm^2; SM0: Sanitised mesclun control; SM100: Sanitised mesclun treated with 100 mJ/cm^2

The lightness of all four UV treated and non-treated samples decreased over the storage period of 12 days. For UM0, the lightness increased gradually from 98.80 to 108.55 from day 0 to day 8 but, a sudden drop in the lightness (95.31) was observed on the last day 12. In case of UM100, the lightness decreased gradually from 101.26 to 87.75 from day 0 to day 12.

There was a very slight change in the lightness of the leaves of sample SM0 from 97.8 to 96.46 over the 12 days storage period whereas for SM100, there was a significant reduction in lightness from 96.04 to 88.89.

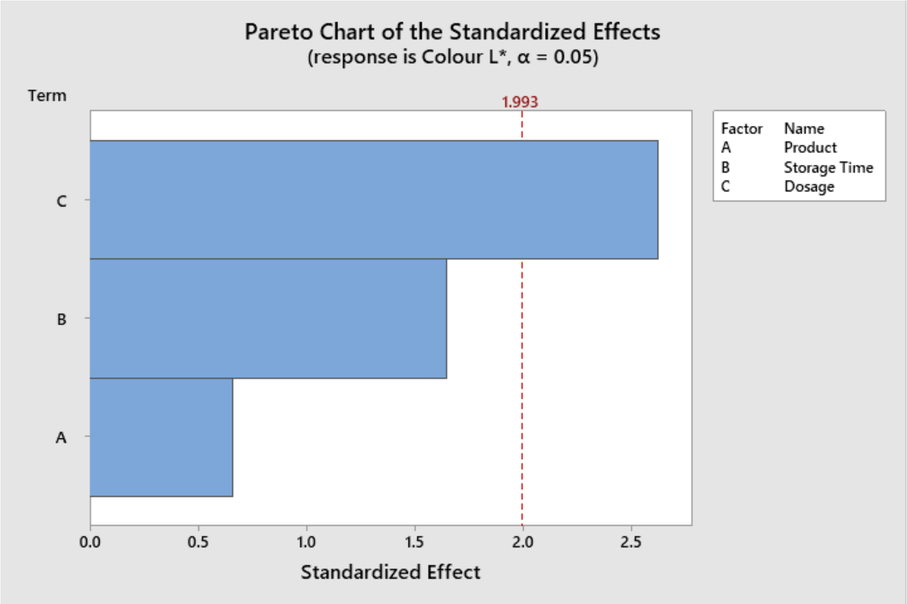


Figure 4.28 Pareto Chart of standardised effects for L* (lightness) of mesclun
Note: A: Product; B: Storage time; C: UV-C dosage

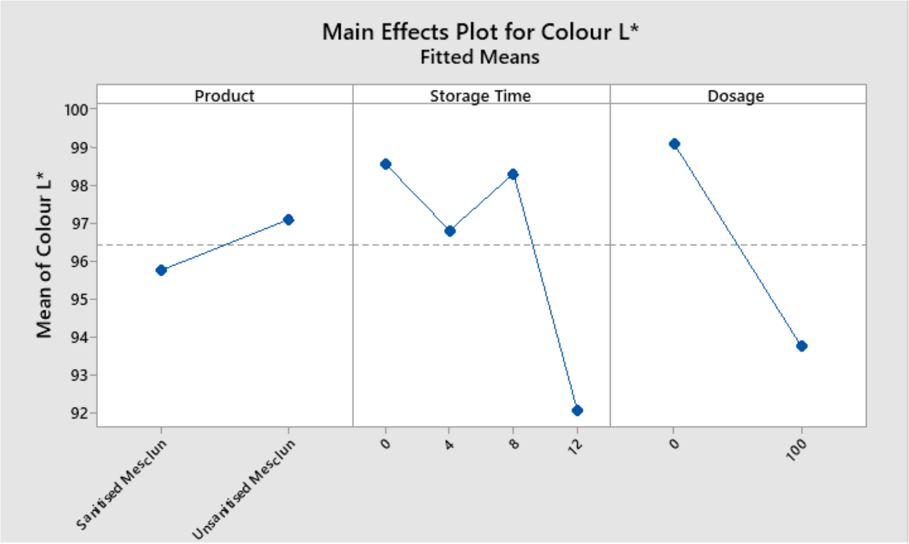


Figure 4.29 Main effect plot for L* (lightness) of mesclun
Note: A: Product; B: Storage time; C: UV-C dosage

Only the dosage 0 and 100 mJ/cm^2 had significant effect ($p<0.05$) on the lightness (L*) of mesclun whereas, the products – sanitised and unsanitised mesclun and the storage period of 12 days had no major significance on the Hunter's L* vale ($p>0.05$).

The sanitised mesclun was less light than unsanitised mesclun. The lightness of mesclun samples decreased from day 0 to 4 and increased from day 4 to 8. The mean lightness highly decreased from day 8 to day 12. The lightness for UV-C treated samples was significantly lower than non-UV-C treated control samples.

- **Greenness**

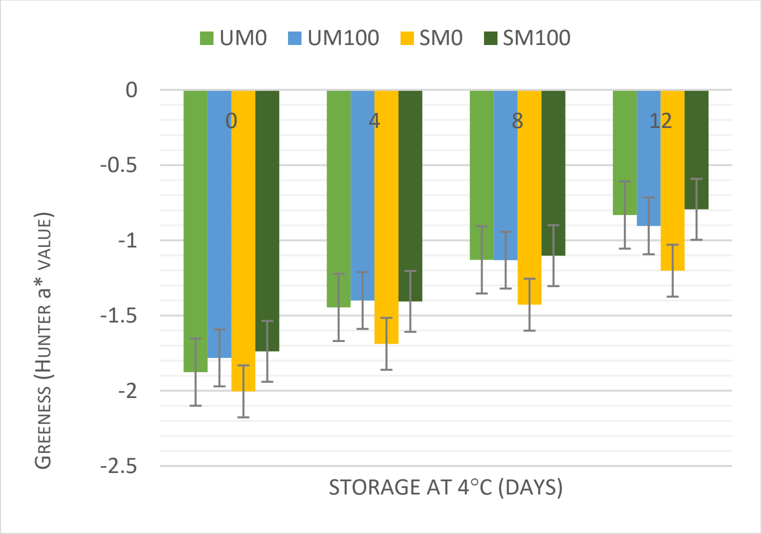


Figure 4.30 Changes in the Hunter a* (greenness) value of UV-C treated and untreated sanitised and unsanitised mesclun during storage at 4° C for 12 days

Note: UM0: Unsanitised mesclun control; UM100: Unsanitised mesclun treated with 100 mJ/cm^2; SM0: Sanitised mesclun control; SM100: Sanitised mesclun treated with 100 mJ/cm^2

The greenness of all the mesclun samples decreased during the storage for 12 days at 4° C. SM100 had the highest drop in greenness from -1.73 to -0.79 and SM0 has the least reduction of greenness from -2.0 to -1.2. Both unsanitised mesclun UM0 and UM100 had gradual decrease from -1.87 to -0.83 and from -1.78 to -0.9 respectively.

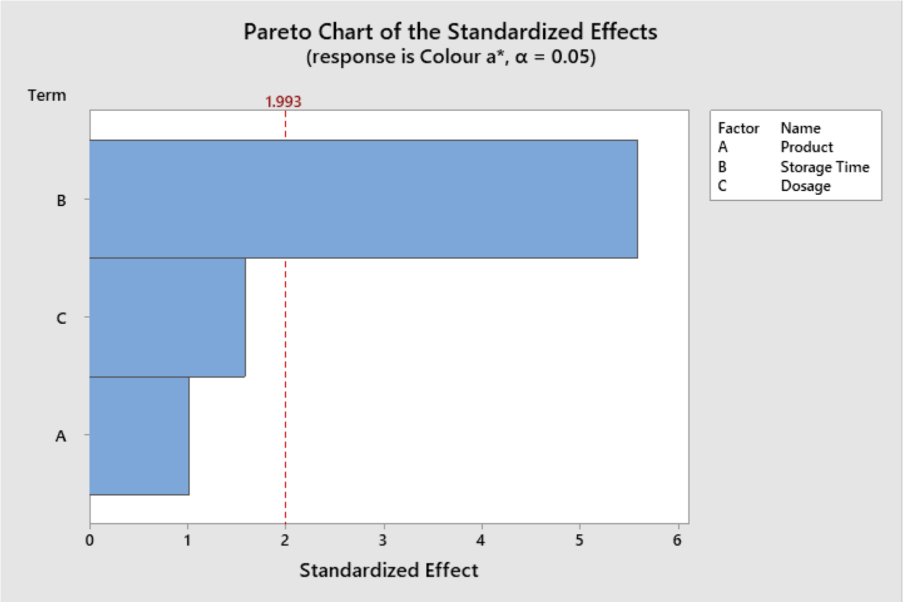


Figure 4.31 Pareto Chart of standardised effects for a* of mesclun
Note: A: Product; B: Storage time; C: UV-C dosage

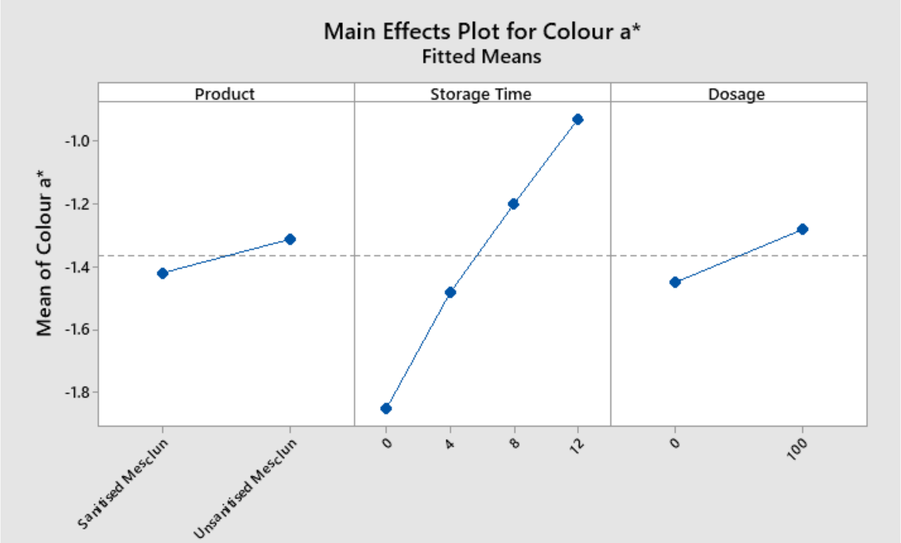


Figure 4.32 Main effect plot for a* (greenness) of mesclun
Note: A: Product; B: Storage time; C: UV-C dosage

The Pareto chart in figure 4.31 represents that the factor that highly affected the a* value (greenness) was the storage time ($p<0.05$). Product type and dosage did not have a strong effect on the greenness ($p>0.05$). In figure 4.32 it was observed that not significant difference was observed between sanitised and unsanitised mesclun and 0 and 100 mJ/cm^2. However, a significant gradual decrease in the greenness was observed over the storage period of 12 days.

- **Yellowness**

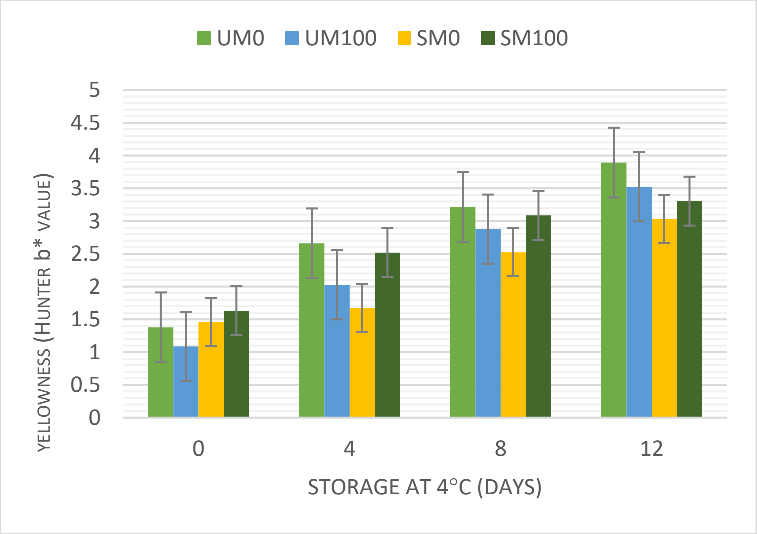


Fig 4.33 Changes in the Hunter b* (yellowness) value of UV-C treated and untreated sanitised and unsanitised mesclun during storage at 4° C for 12 days

Note: UM0: Unsanitised mesclun control; UM100: Unsanitised mesclun treated with 100 mJ/cm^2; SM0: Sanitised mesclun control; SM100: Sanitised mesclun treated with 100 mJ/cm^2

The yellowness of all the four mesclun samples increased over the 12 days shelf-life period. UM0 had the highest increase from 1.37 to 3.89 followed by UM100 from 1.08 to 3.52, SM100 from 1.63 to 3.30 and SM0 from 1.46 to 3.03.

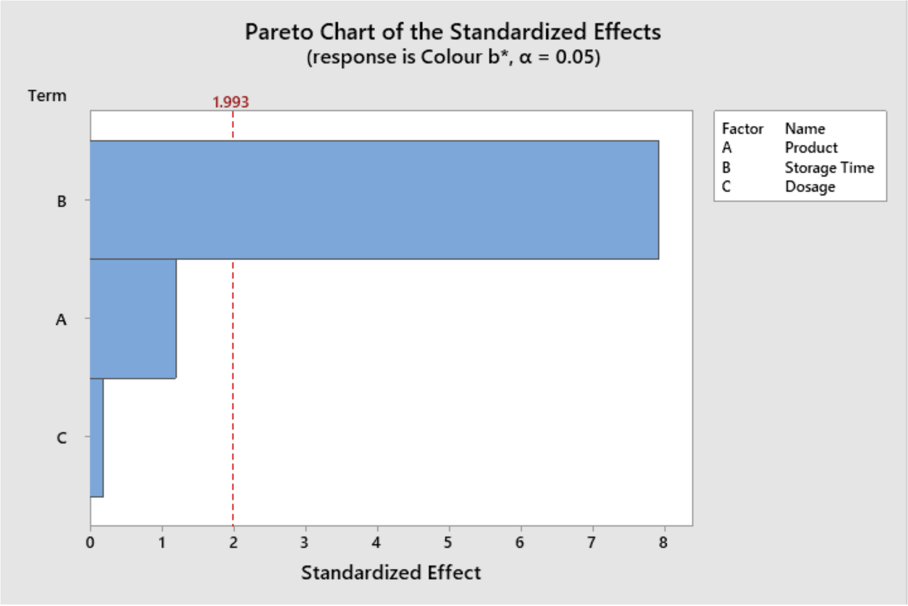


Figure 4.34 Pareto Chart of standardised effects for b* (yellowness) of mesclun
Note: A: Product; B: Storage time; C: UV-C dosage

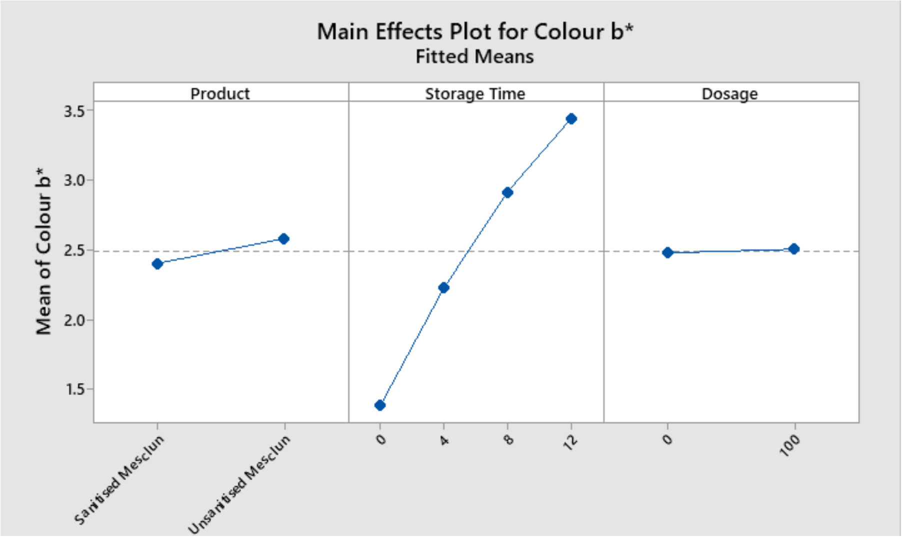


Figure 4.35 Main effect plot for b* (yellowness) of mesclun
Note: A: Product; B: Storage time; C: UV-C dosage

The yellowness (b*) of mesclun samples were affected mainly by the storage time, which had a significant effect ($p<0.05$). The other two factors, product type and dosage did not have any significant effect ($p>0.05$).

The difference in the b* vale between sanitised and unsanitised mesclun was insignificant and in case of dosage also the difference was very minute (p>0.05). During the day 0, 4. 8 and 12, the yellowness of mesclun sample increase steadily.

IV) Rocket

- **Lightness**

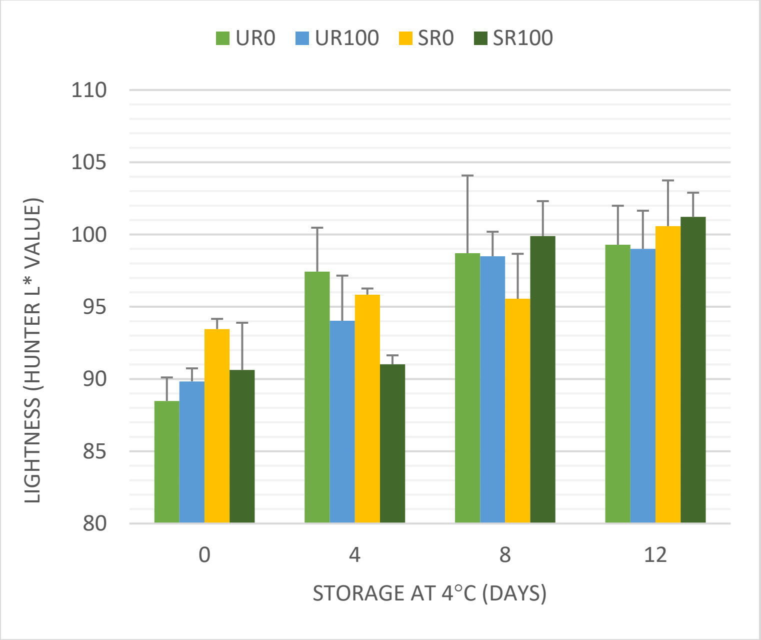


Figure 4.36 Changes in the Hunter L* (lightness) value of UV-C treated and untreated sanitised and unsanitised rocket during storage at 4° C for 12 days

Note: UR0: Unsanitised rocket control; UR100: Unsanitised rocket treated with 100 mJ/cm^2; SR0: Sanitised rocket control; SR100: Sanitised rocket treated with 100 mJ/cm^2

The lightness of all the rocket samples increased during the storage period of 12 days. SR100 had the highest increase from 90.63 to 101.21 whereas, UR100 had the least increase in the lightness from 89.83 to 99.0. UR0 had an increase from 88.48 to 99.29 and SR0 had an increase from 93.45 to 100.58.

These results coincide with the reports by Gutiérrez et al. (2015), surface color parameters for rocket leaves treated with 10, 20 and 30 kJ/m^2 were evaluated during storage. The lightness (L*) increased with time, suggesting incipient yellowing of the leaves at the end of storage for both UV-C-treated and non-treated leaves.

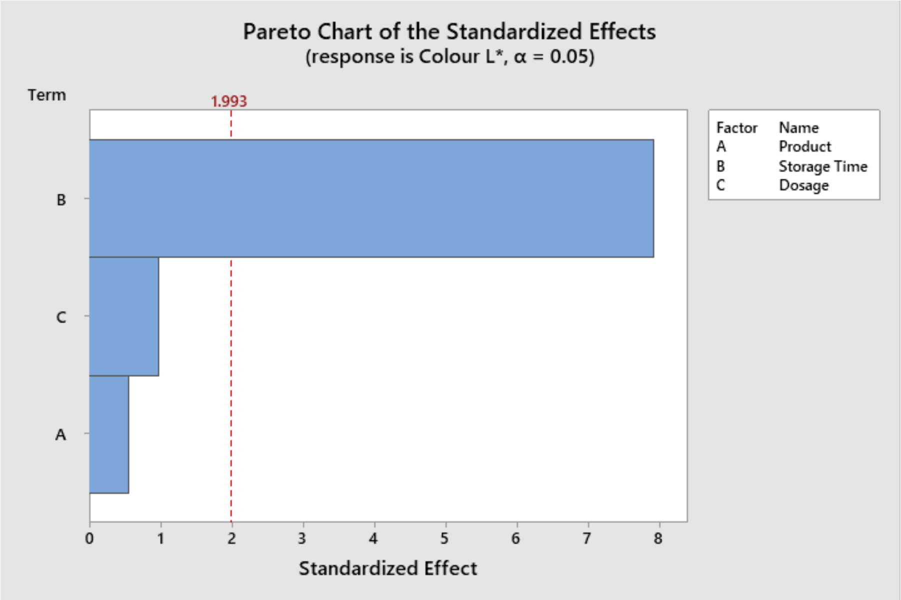


Figure 4.37 Pareto Chart of standardised effects for L* (lightness) of rocket
Note: A: Product; B: Storage time; C: UV-C dosage

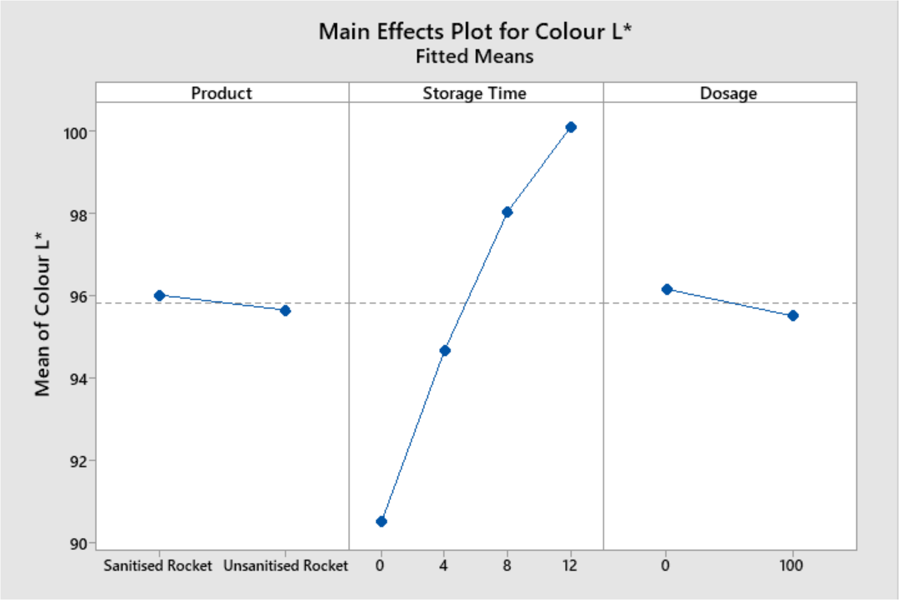


Figure 4.38 Main effect plot for L* (lightness) of rocket
Note: A: Product; B: Storage time; C: UV-C dosage

The data above and the Pareto chart in figure 4.37 reflects that the storage time had significantly major effect on the Hunter's L* value ($p<0.05$). No significant differences were made by the product type and the dosage ($p>0.05$). Lightness of sanitised rocket was slightly higher than the unsanitised rocket. The total cell count increased exponentially from day 0 to day 12 during the storage period. Minute and insignificant differences were observed in the lightness of UV-C treated and non-UV-C treated products.

- **Greenness**

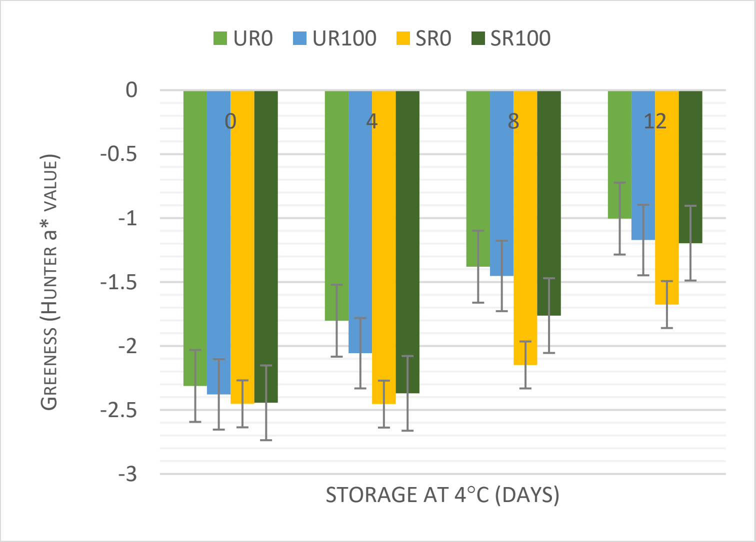


Figure 4.39 Changes in the Hunter a* (greenness) value of UV-C treated and untreated sanitised and unsanitised rocket during storage at 4° C for 12 days

Note: UR0: Unsanitised rocket control; UR100: Unsanitised rocket treated with 100 mJ/cm^2; SR0: Sanitised rocket control; SR100: Sanitised rocket treated with 100 mJ/cm^2

The greenness for all samples decreased during the storage time. SR0 had the least decrease in the greenness and UR0 had the highest decrease in the greenness.

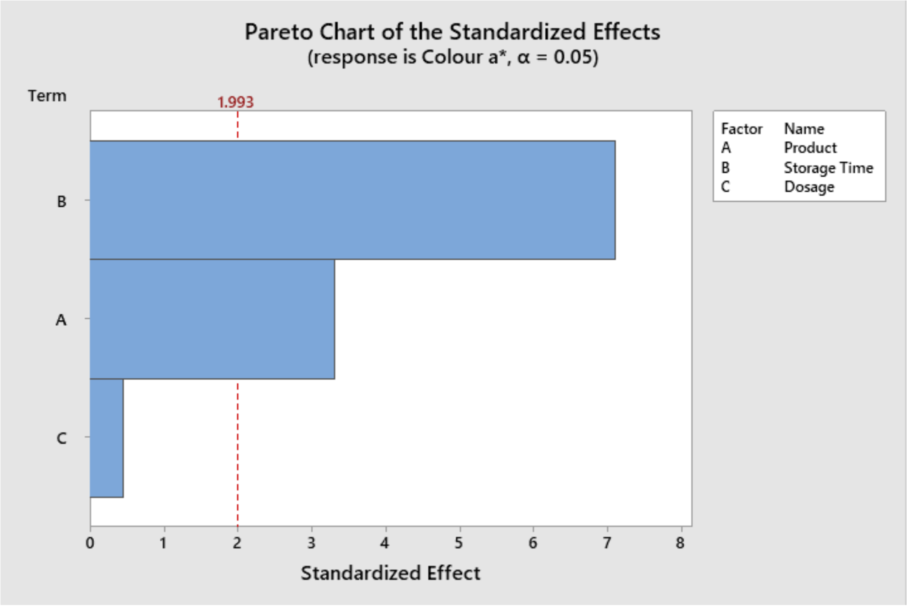


Figure 4.40 Pareto Chart of standardised effects for a* (greenness) of rocket
Note: A: Product; B: Storage time; C: UV-C dosage

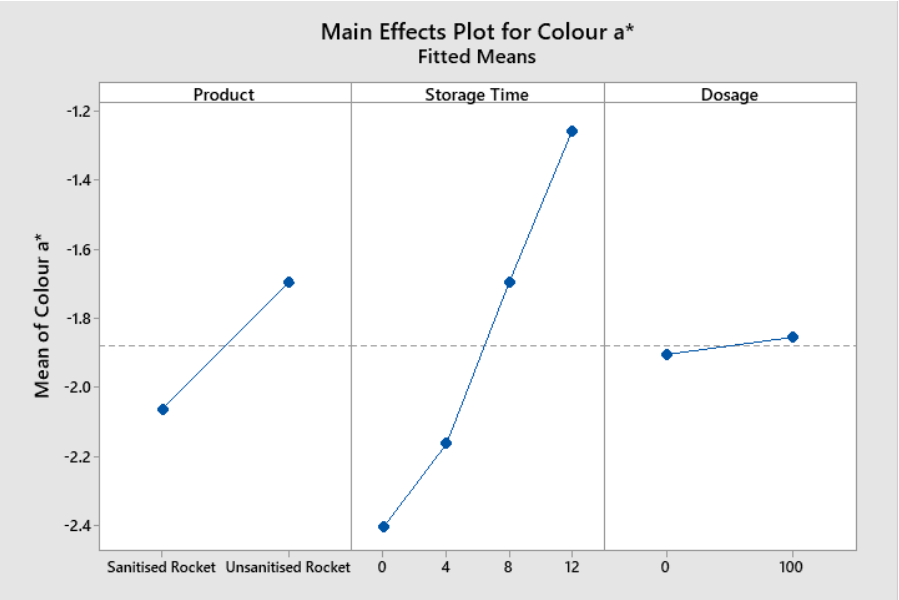


Figure 4.41 Main effect plot for a* (greenness) of rocket
Note: A: Product; B: Storage time; C: UV-C dosage

The factors A and B representing the product and storage time respectively, had significantly major effect ($p<0.05$) on the Hunter's a* value (greenness) whereas, factor C representing dosage, had no significant effect on the greenness ($p>0.05$).

The mean greenness for sanitised rocket was higher to the unsanitised rocket. There was a significant decrease in the greenness from day 0 to 4 but during day 4 to 12, there was a huge decrease in the greenness (a*). The greenness was slightly lesser for UV treated samples than the non-UV treated samples.

- **Yellowness**

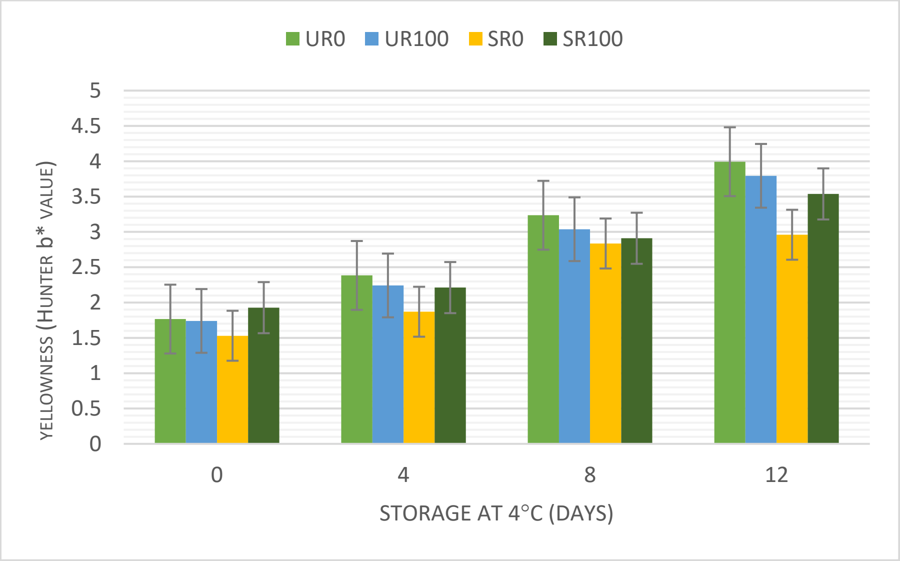


Figure 4.42 Changes in the Hunter b* (yellowness) value of UV-C treated and untreated sanitised and unsanitised rocket during storage at 4° C for 12 days

Note: UR0: Unsanitised rocket control; UR100: Unsanitised rocket treated with 100 mJ/cm^2; SR0: Sanitised rocket control; SR100: Sanitised rocket treated with 100 mJ/cm^2

The yellowness of all the samples raised gradually during 12 days of storage. UR0 had the highest increase in yellowness from 1.76 to 3.99 and SR0 had the minimum increase in the yellowness from 1.53 to 2.96.

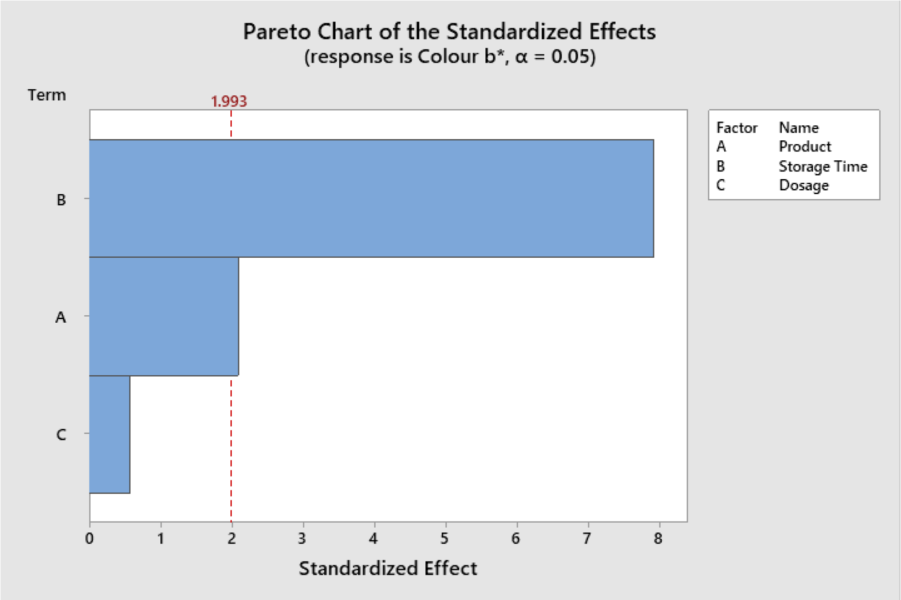


Figure 4.43 Pareto Chart of standardised effects for b* (yellowness) of rocket
Note: A: Product; B: Storage time; C: UV-C dosage

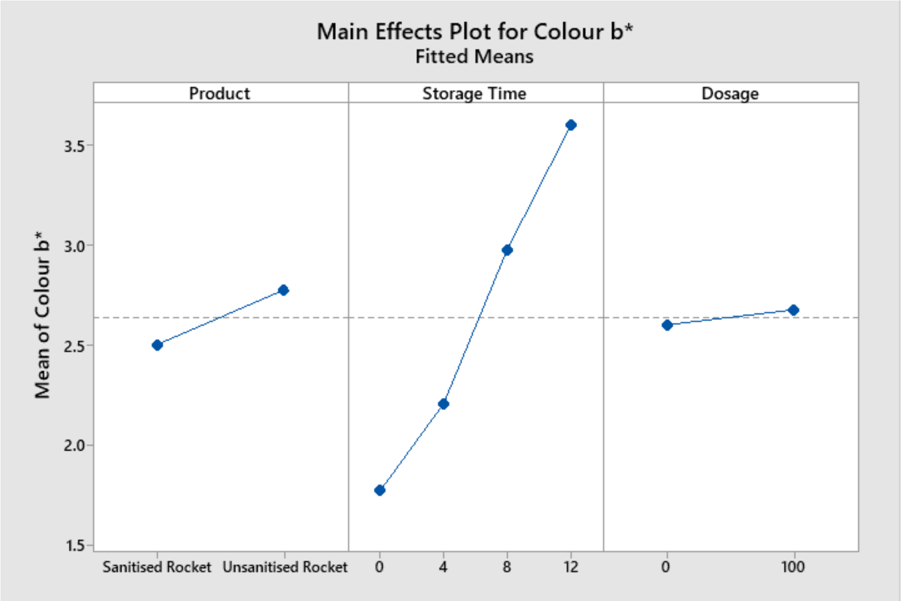


Figure 4.44 Main effect plot for b* (yellowness) of rocket
Note: A: Product; B: Storage time; C: UV-C dosage

The factors that majorly affected the colour yellow of the rocket samples were the product and the storage time ($p<0.05$). The dosage did not make big difference to the yellowness (b*) of rocket.

Mean b* was high for unsanitised rocket and low for sanitised rocket. The yellowness increased from day 0 to 4 of storage and a hike in yellowness was observed during day 8 and 12. The difference in yellowness between 0 mJ/cm^2 and 100 mJ/cm^2 was insignificant.

4.3.3 Weight Analysis

I) Spinach

Percentage weight loss of UV-C treated and untreated sanitised and unsanitised Spinach over the storage period of 12 days at 4° C

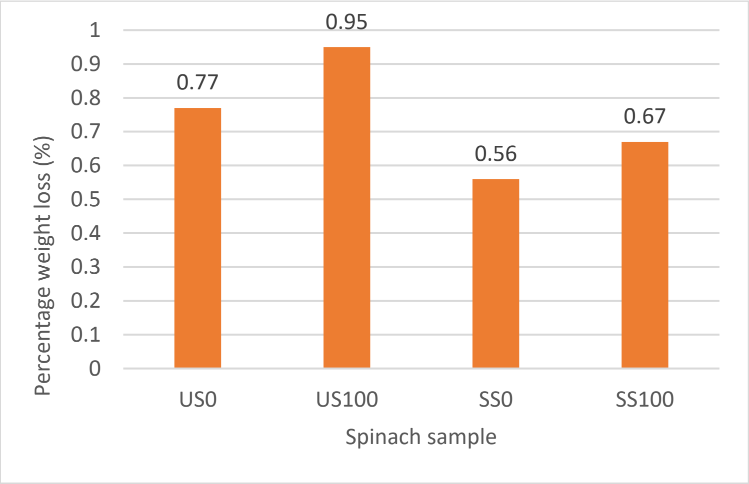


Figure 4.45 Percentage weight loss (%) of UV-C treated and untreated sanitised and unsanitised Spinach over the storage period of 12 days at 4° C

Note: US0: Unsanitised spinach control; US100: Unsanitised spinach treated with 100 mJ/cm^2; SS0: Sanitised spinach control; SS100: Sanitised spinach treated with 100 mJ/cm^2

Figure 4.45 represents the percentage weight loss of sanitised and unsanitised, UV treated and non-treated spinach samples. It can be observed that the percentage weight loss is highest for US100 (0.95) followed by US0 (0.77), SS100 (0.67) and SS0 (0.56). This is similar to the study by Gogo et al. (2017) which reports fresh weight loss of African nightshade significantly increased with the increase in UV-C application dosage, with the control leaves having significantly lower weight loss.

II) Kale

Percentage weight loss of UV-C treated and untreated sanitised and unsanitised kale over the storage period of 12 days at 4° C

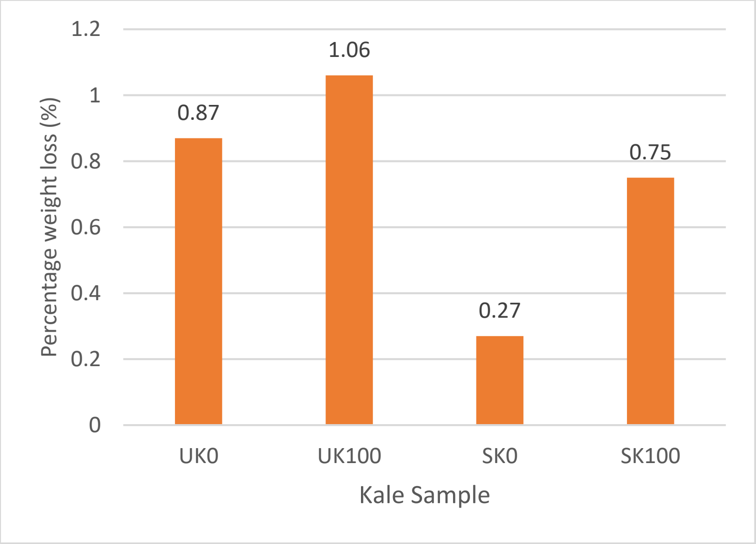


Figure 4.46 Percentage weight loss of UV-C treated and untreated sanitised and unsanitised Spinach over the storage period of 12 days at 4° C

Note: UK0: Unsanitised kale control; UK100: Unsanitised kale treated with 100 mJ/cm2; SK0: Sanitised kale control; SK100: Sanitised kale treated with 100 mJ/cm2

The above u represents percentage weight loss of kale samples and it was observed that UK100 had the highest percentage of weight loss of 1.06% followed by UK0 (0.87%), SK100 (0.75%) and SK0 (0.27%). Our results are corroborated by Karasahin et al. (2005) who observed a higher weight loss in eggplants treated with UV-C at 3.6 kJ/m^2, following hot water treatment compared to the control. In another study, Lemoine et al. (2008) observed no effect on weight loss when broccoli was UV-C treated at 5, 8, and 10 kJ/m^2 UV-C compared to the control.

III) Mesclun

Percentage weight loss of UV-C treated and untreated sanitised and unsanitised kale over the storage period of 12 days at 4° C

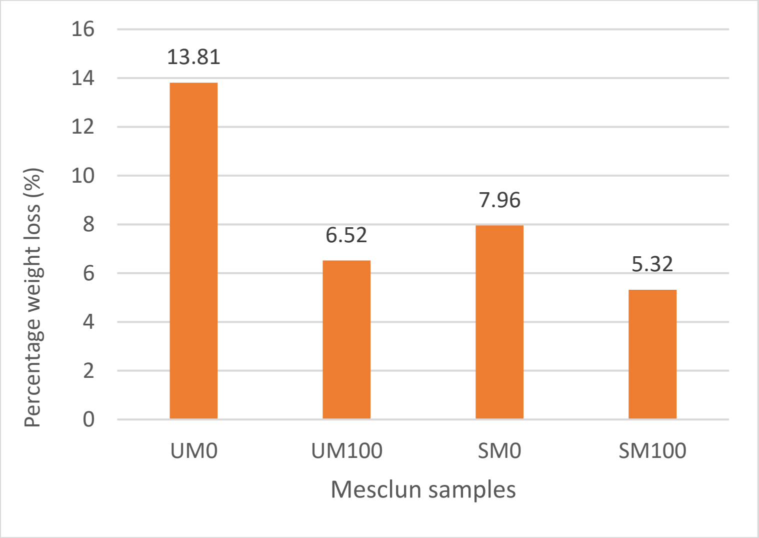


Figure 4.47 Percentage weight loss of UV-C treated and untreated sanitised and unsanitised Spinach over the storage period of 12 days at 4° C

Note: UM0: Unsanitised mesclun control; UM100: Unsanitised mesclun treated with 100 mJ/cm2; SM0: Sanitised mesclun control; SM100: Sanitised mesclun treated with 100 mJ/cm2

The above Figure 4.47 shows the percentage weight loss in sanitised and unsanitised, UV treated and non-treated mesclun during 12 days storage at 4° C and it can be observed that UM0 had highest percentage of weight loss of 13.81% followed by SM0 (7.96), UM100 (6.52) and SM100 (5.32). The data obtained were similar to the findings reported by Moreno et al. (2017) in which UV-C treated carambola showed lower weight loss than control fruit after both 14 and 21 days.

Manzocco and Nicoli suggested that UV-C irradiation favoured the formation of a thin dried layer in the cut surface which may increase the resistance to water flow. However, the reduction in weight loss may have resulted from improved maintenance of tissue integrity in UV treated fruit (Manzocco & Nicoli, 2015).

IV) Rocket

Percentage weight loss of UV-C treated and untreated sanitised and unsanitised rocket over the storage period of 12 days at 4° C

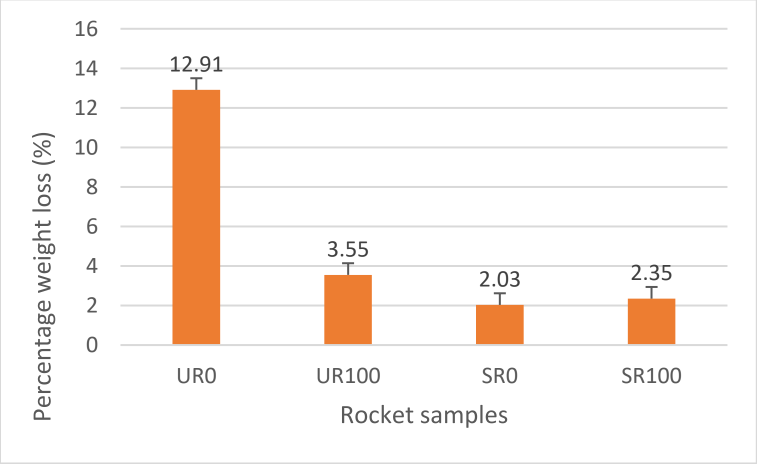


Figure 4.48 Percentage weight loss of UV-C treated and untreated sanitised and unsanitised rocket over the storage period of 12 days at 4° C

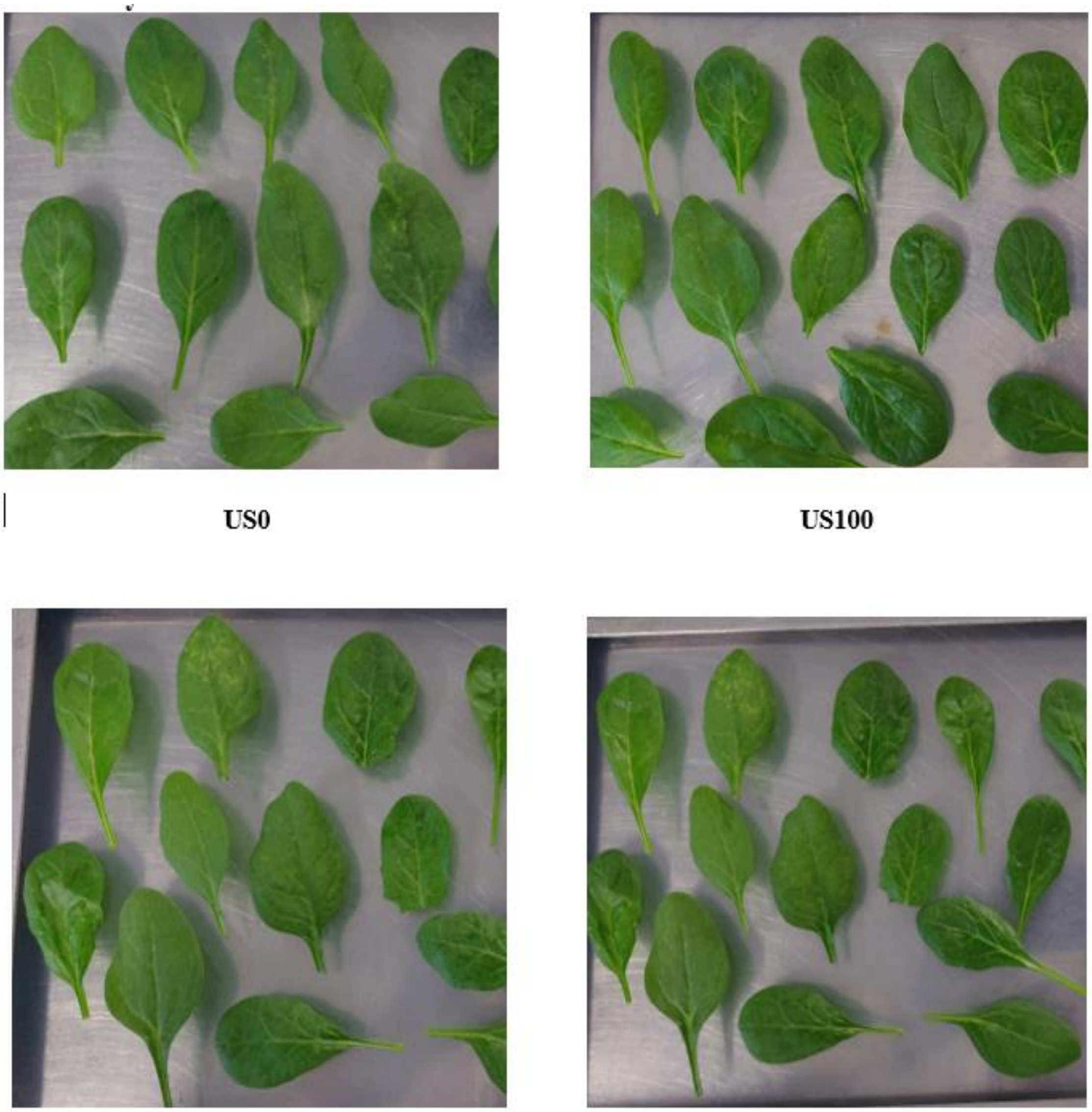
Note: UR0: Unsanitised rocket control; UR100: Unsanitised rocket treated with 100 mJ/cm^2; SR0: Sanitised rocket control; SR100: Sanitised rocket treated with 100 mJ/cm^2

The figure above illustrates the percentage weight loss in sanitised and unsanitised, UV treated and non-treated rocket over 12 days storage at 4° C. It was observed that UR0 had highest percentage of weight loss of 12.91% followed by UR100 (3.55%), SR100 (2.35) and SR0 (2.03). Weight loss in postharvest crops during storage may be attributed to loss of water through transpiration or to loss of substrates (sugar and organic acids) through respiration. Similar results were also observed in UV-treated sweet potatoes (Wu, 1995). Maharaj, Arul & Nadeau (1999) observed higher weight loss in UV-treated tomato fruits than in the untreated control (Maharaj, Arul & Nadeau, 1999).

4.3.4 Sensory Analysis

I) Spinach

- **Spinach Day 0**



US0

US100

SS0

SS100

Figure 4.49 Sanitised and Unsanitised, UV treated and non-treated spinach on day- 0

Note: US0: Unsanitised spinach control; US100: Unsanitised spinach treated with 100 mJ/cm^2; SS0: Sanitised spinach control; SS100: Sanitised spinach treated with 100 mJ/cm^2

On day 0 of sensory evaluation of sanitised and unsanitised UV-C treated and untreated spinach, all the four samples of unsanitised non-UV-C treated spinach (US0), unsanitised UV-C treated (US100), sanitised non-UV-C treated (SS0) and sanitised UV-C treated (SS100) were fresh, healthy and were firm in texture with no sign of dehydration, decay and browning.

The UV-C treated US100 and SS100 and a glossy appearance which enhanced the colour of the spinach leaves whereas the non-UV-C treated leaves did not display the shiny appearance. The non-UV treated SS0 and US0 were less appealing as they looked slightly less fresh when compared to UV treated SS100 and US100. All the spinach samples had good flavour, juiciness and fresh aroma.

- **Spinach Day 4**

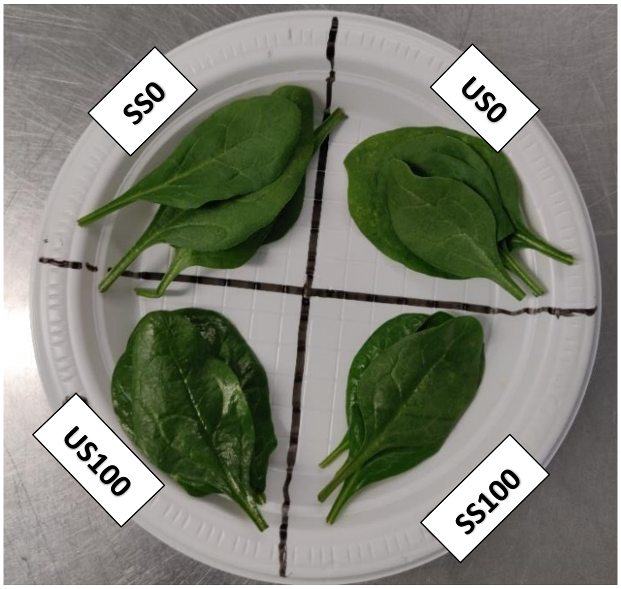


Figure 4.50 Sanitised and Unsanitised, UV treated and non-treated spinach on day- 4
Note: US0: Unsanitised spinach control; US100: Unsanitised spinach treated with 100 mJ/cm^2; SS0: Sanitised spinach control; SS100: Sanitised spinach treated with 100 mJ/cm^2

On day 4 of sensory evaluation of sanitised and unsanitised UV-C treated and untreated spinach, the UV-C treated US100 and SS100 were darker, shinier in appearance as compared to non-UV-C. The appearance of US100 and SS100 were more appealing to people.

Sanitised non-UV-C treated and UV-C treated spinach, SS0 and SS100 were less crunchy in comparison to unsanitised non-UV-C treated and UV-C treated spinach, US0 and US100. All the four samples looked fresh, had firm texture and stable structure.

Among the four samples, SS100 was more preferred based on juiciness and flavour. No specific aroma was observed for any of the samples. No changes in the colour or decay were observed in any of the spinach leaf samples.

- **Spinach Day 8**

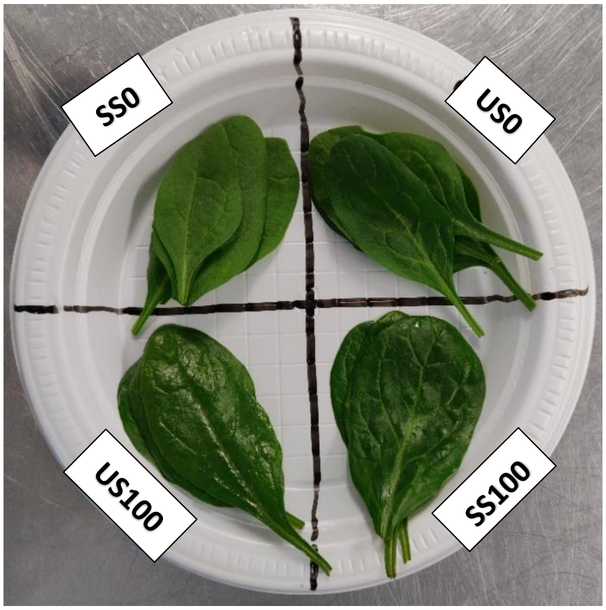


Figure 4.51 Sanitised and Unsanitised, UV treated and non-treated spinach on day- 8
Note: US0: Unsanitised spinach control; US100: Unsanitised spinach treated with 100 mJ/cm^2; SS0: Sanitised spinach control; SS100: Sanitised spinach treated with 100 mJ/cm^2

On day 8 of sensory evaluation of sanitised and unsanitised UV-C treated and untreated spinach, all the four samples appeared to be fresh but the non-UV treated samples appeared to be dry and pale in appearance whereas the UV treated samples appeared shiny and glossy. The appearance and colour of the UV treated US100 and SS100 was preferred over non-UV treated US0 and SS0.

The texture of US100 and SS100 was soft and not very stable whereas the texture for US0 and SS0 was rough and firm. US0 and SS0 were crunchier than US100 and SS100.

The taste, flavour of juiciness of US100 and SS100 was more appealing over US0 and SS0 but US100 was the juiciest among all.

No visible dehydration or injuries were observed in any of the samples. There was no sign of decay and browning of the leaves.

- **Spinach Day 12**

Figure 4.52 Sanitised and Unsanitised, UV treated and non-treated spinach on day- 12
Note: US0: Unsanitised spinach control; US100: Unsanitised spinach treated with 100 mJ/cm^2; SS0: Sanitised spinach control; SS100: Sanitised spinach treated with 100 mJ/cm^2

On day 12 of sensory evaluation of sanitised and unsanitised UV-C treated and untreated spinach, slight discolouration of the leaves can be observed. The non-UV treated leaves, US0 and SS0 appeared to be lighter in colour whereas the UV treated leaves, US100 and SS100 appeared darker in appearance. The texture of both the US0 and SS0 were firm and stable whereas the texture of US100 and SS100 were loose and sticky. Dehydration was observed in the UV treated leaves which cased shrivelling and made them look soggy.

As reported by Artés-Hernández et al. (2009), leaves treated with 7.94 and 11.35 kJ/m^2 UV-C showed dehydration symptoms that exceeded the limit of usability. This was explained by the cellular damage caused where membrane permeability may have been altered (Artés-Hernández et al., 2009).

There was no specific aroma observed in any of the samples. Among all the four samples, US100 was the juiciest and the flavour of SS100 was preferred. US100 had a bitter after taste in comparison to other samples. No decay or browning was observed in any of the leaves. These results were in contrast to the findings by Artés-Hernández et al. where, severe browning was detected after 10 days in UV-C treated leaves stored at 5 and 8 °C, while control leaves showed moderate to severe values in both cases and UV-C treated and control leaves showed unmarketable moderate to severe off-odours after 13 days at both storage temperatures (Artés-Hernández et al., 2009).

The reports by Martínez-Sánchez et al., suggested that spinach showed loss of crispiness and flavor along with reduction in the overall quality of UV treated and the control samples. However, the overall quality of spinach was above the threshold of commercial acceptability in all treatments. During the postharvest storage, the sensorial quality decreased in the UV-C treated samples (Martínez-Sánchez et al., 2019).

II) Kale

- **Kale Day 0**

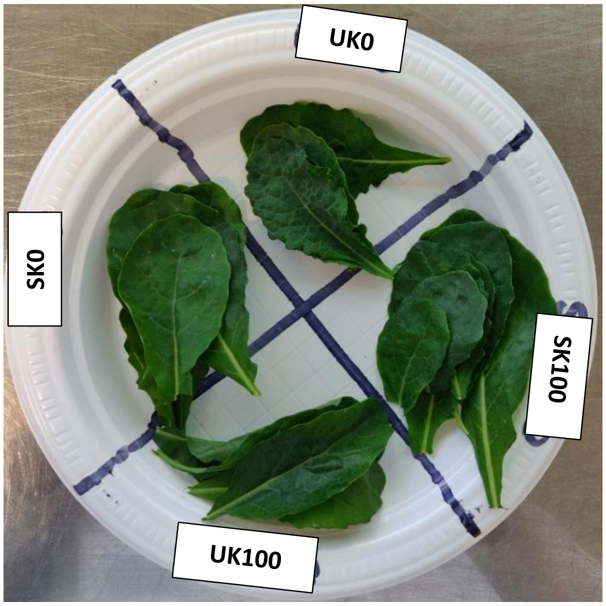


Figure 4.53 Sanitised and Unsanitised, UV treated and non-treated kale on day- 0
Note: UK0: Unsanitised kale control; UK100: Unsanitised kale treated with 100 mJ/cm^2; SK0: Sanitised kale control; SK100: Sanitised kale treated with 100 mJ/cm^2

On day 0 of sensory evaluation of sanitised and unsanitised UV treated and untreated kale, all the leaf samples, UK0, UK100, SK0 and SK100 looked healthy, green, fresh and appetizing. There were no signs of any decolouration, decay, injury or dehydration.

All the leaves appeared to be in good condition with stable structure and rough and crunchy texture. No differences were observed in the appearance of all four-leaf samples.

It was observed that the UV-C treated UK100 had a bitter after taste whereas UK0, SK0 and SK100 were more accepted. SK100 was most preferred in terms of juiciness and flavour. No specific aroma was observed in any of the samples.

- **Kale Day 4**

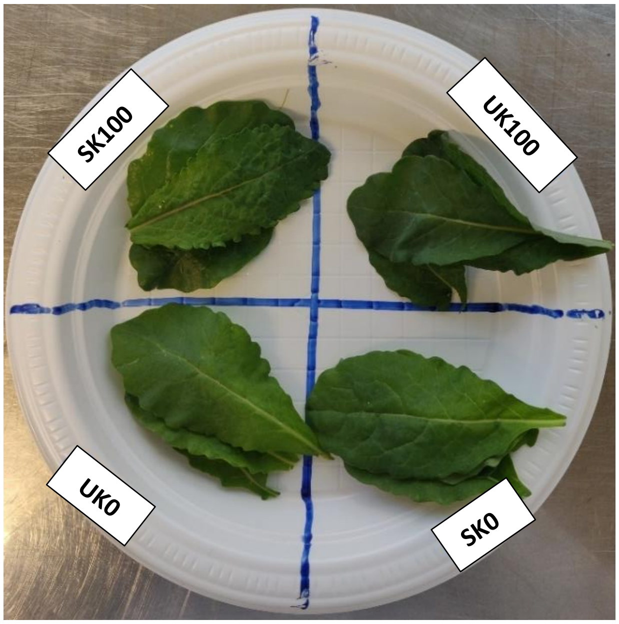


Figure 4.54 Sanitised and Unsanitised, UV treated and non-treated kale on day- 4
Note: UK0: Unsanitised kale control; UK100: Unsanitised kale treated with 100 mJ/cm^2; SK0: Sanitised kale control; SK100: Sanitised kale treated with 100 mJ/cm^2

On day 4 of sensory evaluation of sanitised and unsanitised UV treated and untreated kale, no changes in the colour of the leaves were observed. The texture and firmness of all the four samples, UK0, UK100, SK0 and SK100 were crispy and stable but UK0 appeared to be dry in appearance. Overall, all the leaves looked fresh.

The UV treated UK100 and SK100 were observed to be slightly bitter in taste when compared to the non-UV treated UK0 and SK0. The juiciness for all the four leaves was liked very much.

- **Kale Day 8**

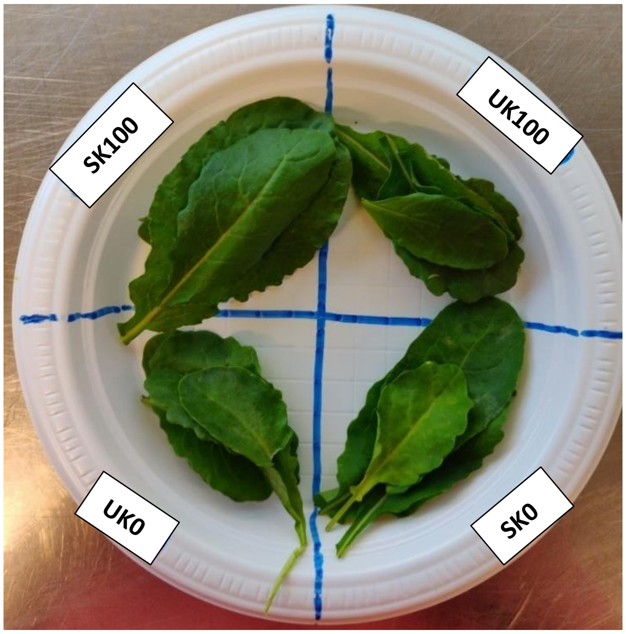


Figure 4.55 Sanitised and Unsanitised, UV treated and non-treated kale on day- 8
Note: UK0: Unsanitised kale control; UK100: Unsanitised kale treated with 100 mJ/cm^2; SK0: Sanitised kale control; SK100: Sanitised kale treated with 100 mJ/cm^2

On day 8 of sensory evaluation of sanitised and unsanitised UV treated and untreated kale, the non-UV treated UK0 appeared to be dry and SK0 seemed stale. Both UK0 and SK0 looked lighter and yellowish whereas no visible discolouration or damage were observed for UK100 and SK100.

All the four leaves had stable structures with no signs of dehydration or sogginess. The flavour and juiciness of UK100 was liked the most followed by UK0, SK100 and SK0. UK 100 was most liked based on the colour, texture and flavours.

- **Kale Day 12**

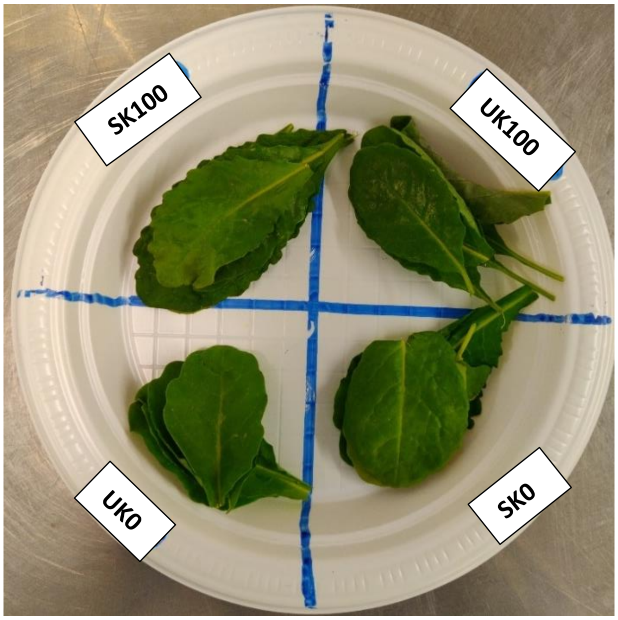


Figure 4.56 Sanitised and Unsanitised, UV treated and non-treated kale on day- 12
Note: UK0: Unsanitised kale control; UK100: Unsanitised kale treated with 100 mJ/cm^2; SK0: Sanitised kale control; SK100: Sanitised kale treated with 100 mJ/cm^2

On the last day of the shelf life of sanitised and unsanitised UV treated and untreated kale, all the samples had intact structure without any damages or wilting. UK0 looked a little dry. SK0 and UK0 appeared to be lighter in colour.

SK0 was bitter in flavour compared to other three samples. No specific aroma was observed in SK0, SK100 and UK100 but, UK0 had slightly unpleasant smell.

The overall texture of SK0 was liked the most whereas, the overall flavour and juiciness of SK100 was liked very much.

III) Mesclun

- **Mesclun Day 0**

Figure 4.57 Sanitised and Unsanitised, UV treated and non-treated mesclun on day- 0
Notes: UM0: Unsanitised mesclun control; UM100: Unsanitised mesclun treated with 100 mJ/cm^2; SM0: Sanitised mesclun control; SM100: Sanitised mesclun treated with 100 mJ/cm^2

On day 0 of sensory analysis of UV treated and untreated sanitised and unsanitised mesclun, all the four sample UM0, UM100, SM0 and SM100 looked very fresh, healthy and in great condition. Appearance wise all the four samples looked similar. The texture for all the samples were firm, stable and had no physical damage. The colour of all the leaves were liked extremely and had no aroma. Juiciness of SM0 was liked the most and the flavour of SM100 was liked most. UM0 was slightly bitter in taste when compared to other samples.

- **Mesclun Day 4**

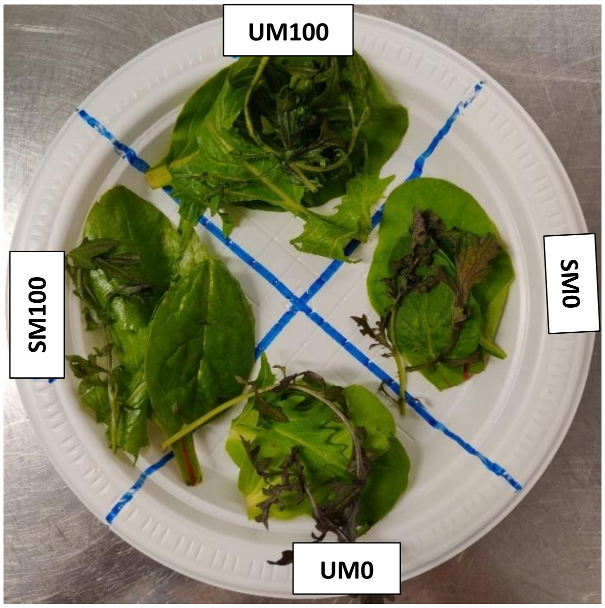


Figure 4.58 Sanitised and Unsanitised, UV treated and non-treated mesclun on day- 4
Note: UM0: Unsanitised mesclun control; UM100: Unsanitised mesclun treated with 100 mJ/cm^2; SM0: Sanitised mesclun control; SM100: Sanitised mesclun treated with 100 mJ/cm^2

On day 4 of sensory analysis of UV treated and untreated sanitised and unsanitised mesclun, the UV treated samples, US100 and SS100 had glossy and shiny surface and looked darker as compared to the non-UV treated US0 and SS0.

In UM0, slight browning and off odour was observed. In SM0, slight dehydration was observed. In the US100 and SS100 samples, the firmness was little less.

The flavour and juiciness of UM100 was liked the most followed by SM100, SM0 and UM0. There was no sign of decay in any of the samples.

- **Mesclun Day 8**

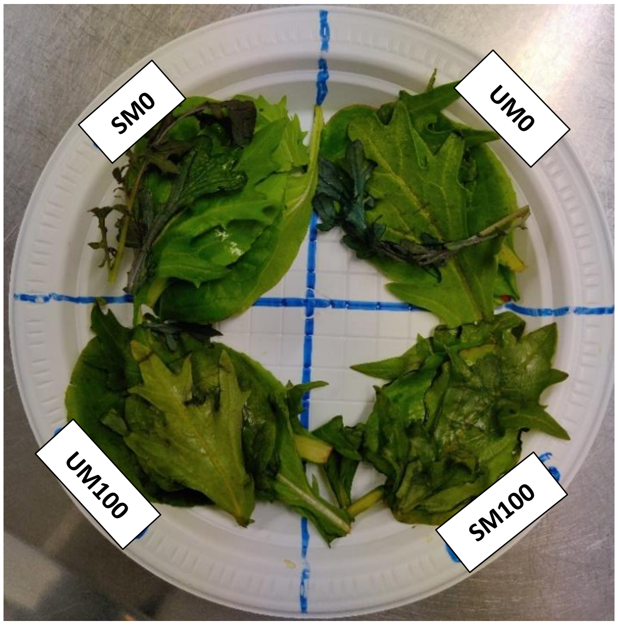


Figure 4.59 Sanitised and Unsanitised, UV treated and non-treated mesclun on day- 8
Note: UM0: Unsanitised mesclun control; UM100: Unsanitised mesclun treated with 100 mJ/cm^2; SM0: Sanitised mesclun control; SM100: Sanitised mesclun treated with 100 mJ/cm^2

On day 4 of storage of UV treated and untreated sanitised and unsanitised mesclun, SM0 looked fresh and had no sign of dehydration. Some of its leaves of SM0 were soggy and damaged but no decay or visible colour change was observed. Even though some of the non-UV treated UM0 had firm texture and stability, many of the leaves appeared old due to browning and decaying of the leaves. UM0 had a nonappealing smell.

Both the UV-C treated UM100 and SM100 were visually not appealing. The SM100 was dark in colour and had glossy surface and had browning on the edges. UM100 and SM100 were highly fragile, wilted and dehydrated. The leaves deteriorated and appeared old and non-appetizing.

- **Mesclun Day 12**

Figure 4.60 Sanitised and Unsanitised, UV treated and non-treated mesclun on day- 12
Note: UM0: Unsanitised mesclun control; UM100: Unsanitised mesclun treated with 100 mJ/cm^2; SM0: Sanitised mesclun control; SM100: Sanitised mesclun treated with 100 mJ/cm^2

On day 12 of sensory analysis of UV treated and untreated sanitised and unsanitised mesclun, all the leaves appeared deteriorated except SM0. Most of the leaves of SM0 had no changes in the physical appearance or the texture but some of the leaves were soggy and looked old. The leaves at the bottom of the package were decoloured and decaying was observed in them. Water retention was observed at the bottom of the package.

The UM0 leaves had an unpleasant odour. Most of the leaves were spoiled and decayed. The UM0 leaves were highly dehydrated, loose structured and sticky and it too had water retention at the bottom of the package.

The UV treated UM100 and SM100 were highly dehydrated, sticky, fragile, wilted and shrivelled. No foul smell was observed in both the samples. Browning and decaying were observed in both the samples and both were inedible.

D’hallewin et al. did not consider UV-C to be an effective method compared with either heat treatment or thiaben-dazole, mainly because of the poor visual appearance of the UV-C-treated fruits, even though the level of decay control was significant (less than 50% of the control). The extensive loss of sensory quality observed by D'hallewin et al. may have been due to over exposure. Similar quality losses were also observed in other crops when doses were higher than the hormetic dose (D'hallewin et al., 1993).

IV) Rocket

- **Rocket Day 0**

Figure 4.61 Sanitised and Unsanitised, UV treated and non-treated rocket on day- 0
Note: UR0: Unsanitised rocket control; UR100: Unsanitised rocket treated with 100 mJ/cm^2; SR0: Sanitised rocket control; SR100: Sanitised rocket treated with 100 mJ/cm^2

On day 0 of sensory analysis of sanitised and unsanitised, UV and non-UV treated samples of rocket, all the four samples UR0, UR100, SR0 and SR100 had stable texture and steady firmness. All the leaf samples appeared to be fresh, green and in excellent condition. No traces of dryness, browning or decaying.

Juiciness of SR0 was liked extremely and the flavour of SR100 was liked the most. SR0 tasted least bitter whereas, UR0 had the most bitter taste.

- **Rocket Day 4**

Figure 4.62 Sanitised and Unsanitised, UV treated and non-treated rocket on day- 4
Note: UR0: Unsanitised rocket control; UR100: Unsanitised rocket treated with 100 mJ/cm^2; SR0: Sanitised rocket control; SR100: Sanitised rocket treated with 100 mJ/cm^2

On day 4 of sensory analysis of sanitised and unsanitised, UV and non-UV treated samples of rocket, the UR100 and SR100 samples appeared to have shiny and glossy coating. No changes were observed in the firmness or the texture of the leaves. The colour of UV-C treated samples was more appealing and liked. Slight dehydration and browning were observed in UR0 samples.

The flavours and juiciness of both SR100 and UR100 were liked the most as they had less bitterness. UR0 was the most bitter among all four samples. No specific aroma or odour was observed in any sample.

- **Rocket Day 8**

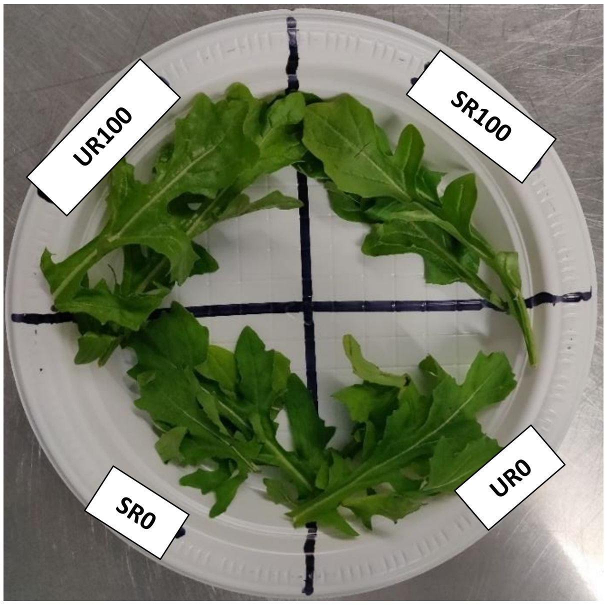


Figure 4.63 Sanitised and Unsanitised, UV treated and non-treated rocket on day- 8
Note: UR0: Unsanitised rocket control; UR100: Unsanitised rocket treated with 100 mJ/cm^2; SR0: Sanitised rocket control; SR100: Sanitised rocket treated with 100 mJ/cm^2

On day 8 of sensory analysis of sanitised and unsanitised, UV and non-UV treated samples of rocket, texture of SR0 was liked the most and UR100 was liked the least. Colour of UV treated SR100 was liked the most when compared to non-UV treated samples. Juiciness of UR0 was preferred over other samples.

Slight discolouration and decaying were observed in UR0 samples. A shiny wax like coating was observed in UR100 and SR100 samples. The non-UV treated UR0 and SR0 samples were bitter in taste whereas SR100 had the least bitterness.

- **Rocket Day 12**

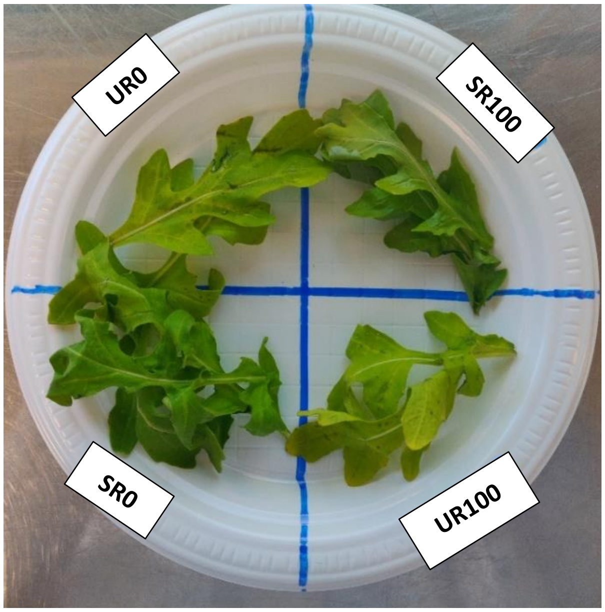


Figure 4.64 Sanitised and Unsanitised, UV treated and non-treated rocket on day- 12
Note: UR0: Unsanitised rocket control; UR100: Unsanitised rocket treated with 100 mJ/cm^2; SR0: Sanitised rocket control; SR100: Sanitised rocket treated with 100 mJ/cm^2

On day 12 of sensory analysis of sanitised and unsanitised, UV and non-UV treated samples of rocket, black spots were observed on both unsanitised UR0 and UR100 samples. Both the samples appeared yellowish in colour. There was no major colour change in SR0 sample whereas, slight discolouration was observed for SR100 samples.

Slight dehydration was observed in all the samples. The degree of browning and decaying was severe in UR0 and UR100. Some of the SR0 leaves were wilted but its flavour was liked the most, followed by SR100 SR100 was liked the juiciest.

Gutiérrez et al. (2015) found that visual color scores had no significant difference between UV-C-treated and untreated samples until day 8. All treatments showed an important decrease in overall appearance at 12 days at 5 ˚C, being unacceptable for fresh consumption. A maximum shelf life of 8 days at 5 ˚C was established for all treatments.

5.0 CONCLUSION

In conclusion, the Phase I study found that UV-C dosages of 100-150 mJ/cm^2 were effective in reducing microbial levels in leafy salads without causing significant damage to their appearance. In Phase II, factors like salad type, sanitization, and UV-C dosage had significant impacts on microbial counts. Sanitized leafy greens and UV-C dosage of 100 mJ/cm^2 significantly reduced microbial counts in the salads and hence, dosage 100 mJ/cm^2 was selected as the optimum dosage. Phase III focused on a shelf-life study of leafy greens treated with a standardized UV-C dosage of 100 mJ/cm^2 compared to untreated controls. Over the 12-day storage period at 4°C, the UV-C treated salads exhibited lower microbial growth compared to untreated salads. Overall, UV-C treatment at 100 mJ/cm^2 shows promise in improving the safety and quality of ready-to-eat salads and extended the shelf life of the salads. This technology has the potential to enhance the safety and quality of ready-to-eat salads, providing consumers with healthier and safer food options. Further research and implementation of UV-C treatment in the food industry could help address foodborne illness concerns and improve food safety standards.

The colour analysis of spinach, kale, mesclun, and rocket samples showed significant changes during storage. UV-C treatment generally led to a decrease in lightness and greenness and an increase in yellowness. Storage time was a critical factor affecting the colour attributes. Further research is needed to optimize UV-C dosage and storage conditions for preserving the colour and quality of leafy greens.

Overall, the weight analysis indicates that UV-C treatment and sanitation have varying effects on weight loss in different leafy greens. The results highlight the complexity of postharvest physiology and responses to UV-C treatment among different varieties of leafy greens. Further research is needed to better understand the underlying mechanisms behind these variations and to optimize UV-C treatment protocols for different leafy greens to minimize weight loss during storage and prolong shelf life.

The sensory evaluation showed that UV-C treatment had positive effects on the appearance, color, and freshness of some leafy greens, but it also led to dehydration and deterioration over time. Further optimization of UV-C treatment parameters and storage conditions is necessary to improve the overall shelf life and sensory qualities of these leafy greens.

Ultimately, UV-C treatment holds promise in enhancing the safety and quality of ready-to-eat salads, providing consumers with healthier and safer food options. Further research and implementation of this technology in the food industry can address foodborne illness concerns and improve food safety standards.

6.0 RECOMMENDATION FOR FUTURE WORK

Based on the results obtained from the study, a few recommendations can be made for future work:

1. One day old samples were used for the study. Further studies can be conducted by examining the effect of UV-C treatment on fresh leafy vegetables obtained directly from the end of production line.

2. Not much information is available on the effects of UV-C treatment on non-inoculated fresh leafy vegetables. Hence, further research is required to enhance the effectiveness of UV-C light treatment.

3. To optimize the UV-C treatment process, the integration of an automated packaging system is recommended. As a result, this approach can significantly enhance the overall quality and safety of the produce.

4. As a dosage of 100 mJ/cm2 resulted in undesirable sensory changes for some leafy greens by the end of the shelf life, further studies are necessary to identify ideal UV-C dosages that preserve sensory quality throughout the shelf life.

5. The microorganisms observed during APC analysis should be examined to identify specific microorganisms surviving on leafy green salads. By identifying and analyzing these bacteria populations, targeted strategies can be developed to maximize the effectiveness of UV-C treatment and ensure enhanced food safety measures.

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8.0 APPENDIX

Appendix A1: Minitb output for Phase II

Table A1: Minitab Design Matrix and Response Data

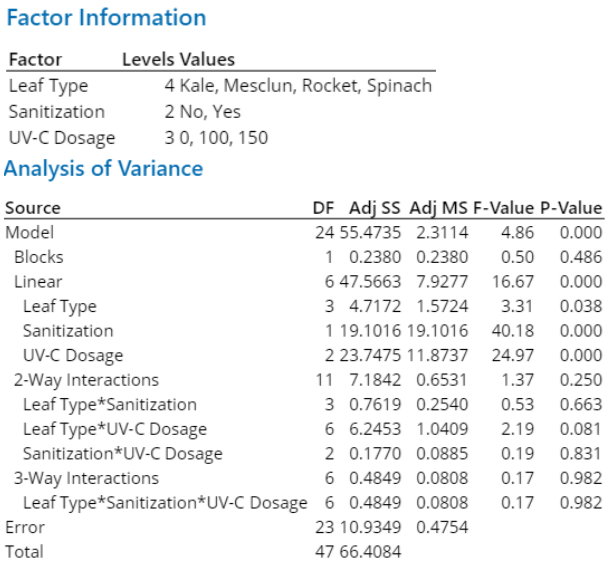
StdOrder	RunOrder	PtType	Blocks	Leaf Type	Sanitized	UV-C Dosage	Yield
31	1	1	2	Kale	No	0	7.21
35	2	1	2	Kale	Yes	100	5.33
37	3	1	2	Mesclun	No	0	7.06
45	4	1	2	Rocket	No	150	5.26
26	5	1	2	Spinach	No	100	6.14
48	6	1	2	Rocket	Yes	150	5.34
40	7	1	2	Mesclun	Yes	0	5.26
39	8	1	2	Mesclun	No	150	5.8
33	9	1	2	Kale	No	150	3.35
34	10	1	2	Kale	Yes	0	5.47
44	11	1	2	Rocket	No	100	4.73
29	12	1	2	Spinach	Yes	100	4.36
32	13	1	2	Kale	No	100	3.57
27	14	1	2	Spinach	No	150	3.9
25	15	1	2	Spinach	No	0	6.29
30	16	1	2	Spinach	Yes	150	3.95
41	17	1	2	Mesclun	Yes	100	4.34
43	18	1	2	Rocket	No	0	6.04
38	19	1	2	Mesclun	No	100	4.5
28	20	1	2	Spinach	Yes	0	5.36
47	21	1	2	Rocket	Yes	100	5.27
36	22	1	2	Kale	Yes	150	3.32
46	23	1	2	Rocket	Yes	0	7.52
42	24	1	2	Mesclun	Yes	150	3.67
21	25	1	1	Rocket	No	150	3.64
15	26	1	1	Mesclun	No	150	4.9
5	27	1	1	Spinach	Yes	100	5.69
24	28	1	1	Rocket	Yes	150	7.03
20	29	1	1	Rocket	No	100	3.33
14	30	1	1	Mesclun	No	100	5
17	31	1	1	Mesclun	Yes	100	5.85
16	32	1	1	Mesclun	Yes	0	6.66
18	33	1	1	Mesclun	Yes	150	5.24
2	34	1	1	Spinach	No	100	6.14
1	35	1	1	Spinach	No	0	6.29
13	36	1	1	Mesclun	No	0	6.89
12	37	1	1	Kale	Yes	150	5.23

8	38	1	1	Kale	No	100	3.65
19	39	1	1	Rocket	No	0	5
3	40	1	1	Spinach	No	150	3.9
10	41	1	1	Kale	Yes	0	6.17
6	42	1	1	Spinach	Yes	150	6.01
22	43	1	1	Rocket	Yes	0	5.67
4	44	1	1	Spinach	Yes	0	4.57
23	45	1	1	Rocket	Yes	100	2.87
11	46	1	1	Kale	Yes	100	2.79
9	47	1	1	Kale	No	150	3.28
7	48	1	1	Kale	No	0	5.64

Note:

Blocks 1 and 2 represent two batches of the salad samples which were supplied on different days; batches were introduced into the model to improve the precision of the results; *industrially sanitised.

Appendix A2: ANOVA of Phase II factors (leaf type, sanitization and dosage)

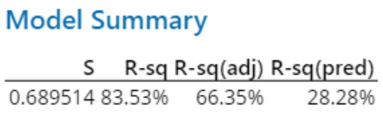


Factor Information

Factor	Levels	Values
Leaf Type	4	Kale, Mesclun, Rocket, Spinach
Sanitization	2	No, Yes
UV-C Dosage	3	0, 100, 150

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	24	55.4735	2.3114	4.86	0.000
Blocks	1	0.2380	0.2380	0.50	0.486
Linear	6	47.5663	7.9277	16.67	0.000
Leaf Type	3	4.7172	1.5724	3.31	0.038
Sanitization	1	19.1016	19.1016	40.18	0.000
UV-C Dosage	2	23.7475	11.8737	24.97	0.000
2-Way Interactions	11	7.1842	0.6531	1.37	0.250
Leaf Type*Sanitization	3	0.7619	0.2540	0.53	0.663
Leaf Type*UV-C Dosage	6	6.2453	1.0409	2.19	0.081
Sanitization*UV-C Dosage	2	0.1770	0.0885	0.19	0.831
3-Way Interactions	6	0.4849	0.0808	0.17	0.982
Leaf Type*Sanitization*UV-C Dosage	6	0.4849	0.0808	0.17	0.982
Error	23	10.9349	0.4754		
Total	47	66.4084			

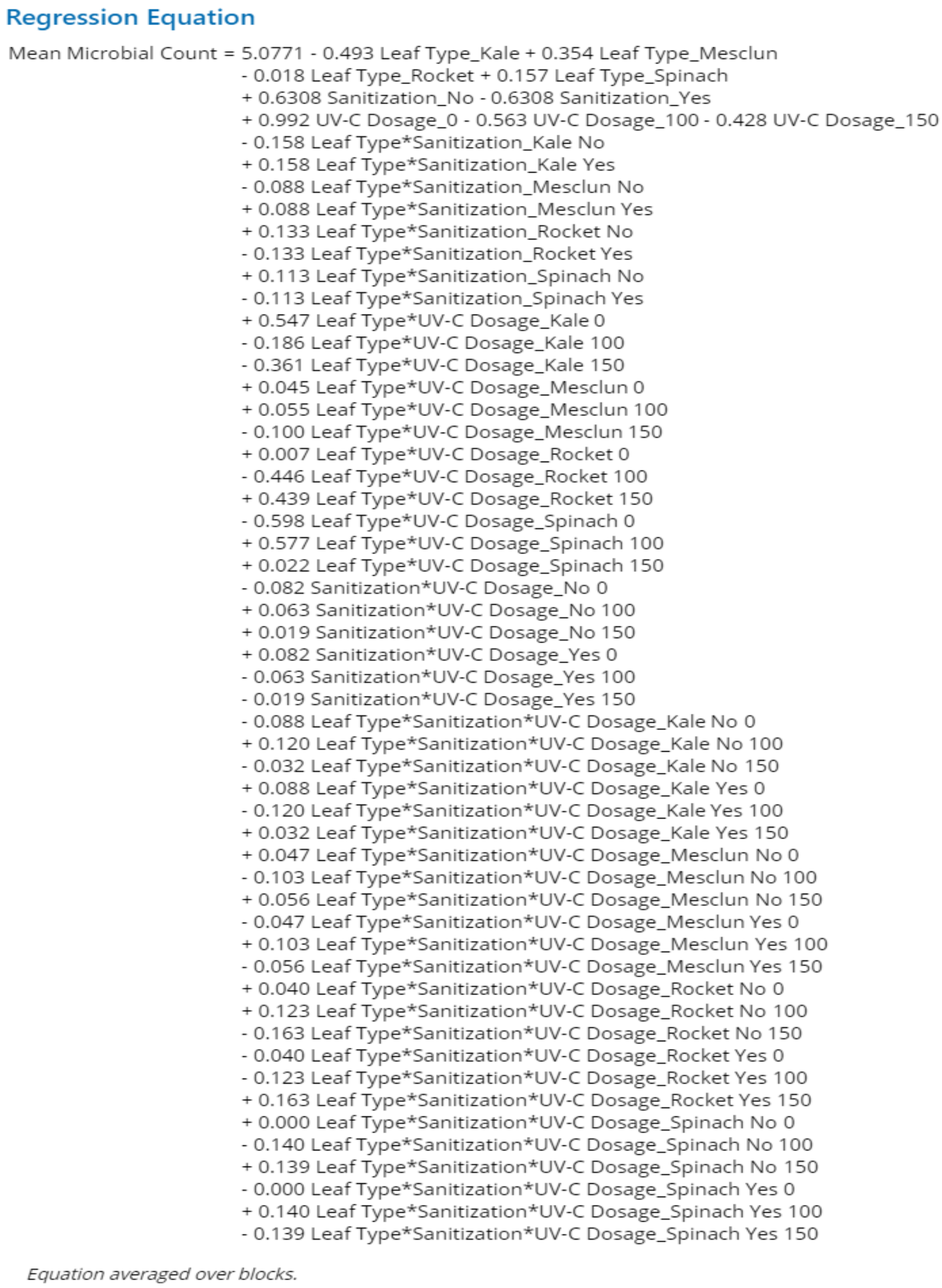


Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.689514	83.53%	66.35%	28.28%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	5.0771	0.0995	51.01	0.000	
Blocks					
1	-0.0704	0.0995	-0.71	0.486	1.00
Leaf Type					
Kale	-0.493	0.172	-2.86	0.009	1.50
Mesclun	0.354	0.172	2.05	0.052	1.50
Rocket	-0.018	0.172	-0.10	0.918	1.50
Sanitization					
No	0.6308	0.0995	6.34	0.000	1.00
UV-C Dosage					
0	0.992	0.141	7.05	0.000	1.33
100	-0.563	0.141	-4.00	0.001	1.33
Leaf Type*Sanitization					
Kale No	-0.158	0.172	-0.92	0.368	1.50
Mesclun No	-0.088	0.172	-0.51	0.613	1.50
Rocket No	0.133	0.172	0.77	0.447	1.50
Leaf Type*UV-C Dosage					
Kale 0	0.547	0.244	2.24	0.035	2.00
Kale 100	-0.186	0.244	-0.76	0.454	2.00
Mesclun 0	0.045	0.244	0.18	0.855	2.00
Mesclun 100	0.055	0.244	0.23	0.823	2.00
Rocket 0	0.007	0.244	0.03	0.978	2.00
Rocket 100	-0.446	0.244	-1.83	0.080	2.00
Sanitization*UV-C Dosage					
No 0	-0.082	0.141	-0.58	0.565	1.33
No 100	0.063	0.141	0.45	0.659	1.33
Leaf Type*Sanitization*UV-C Dosage					
Kale No 0	-0.088	0.244	-0.36	0.722	2.00
Kale No 100	0.120	0.244	0.49	0.628	2.00
Mesclun No 0	0.047	0.244	0.19	0.849	2.00
Mesclun No 100	-0.103	0.244	-0.42	0.677	2.00
Rocket No 0	0.040	0.244	0.17	0.870	2.00
Rocket No 100	0.123	0.244	0.50	0.619	2.00



Regression Equation

```
Mean Microbial Count = 5.0771 - 0.493 Leaf Type_Kale + 0.354 Leaf Type_Mesclun
                       - 0.018 Leaf Type_Rocket + 0.157 Leaf Type_Spinach
                       + 0.6308 Sanitization_No - 0.6308 Sanitization_Yes
                       + 0.992 UV-C Dosage_0 - 0.563 UV-C Dosage_100 - 0.428 UV-C Dosage_150
                       - 0.158 Leaf Type*Sanitization_Kale No
                       + 0.158 Leaf Type*Sanitization_Kale Yes
                       - 0.088 Leaf Type*Sanitization_Mesclun No
                       + 0.088 Leaf Type*Sanitization_Mesclun Yes
                       + 0.133 Leaf Type*Sanitization_Rocket No
                       - 0.133 Leaf Type*Sanitization_Rocket Yes
                       + 0.113 Leaf Type*Sanitization_Spinach No
                       - 0.113 Leaf Type*Sanitization_Spinach Yes
                       + 0.547 Leaf Type*UV-C Dosage_Kale 0
                       - 0.186 Leaf Type*UV-C Dosage_Kale 100
                       - 0.361 Leaf Type*UV-C Dosage_Kale 150
                       + 0.045 Leaf Type*UV-C Dosage_Mesclun 0
                       + 0.055 Leaf Type*UV-C Dosage_Mesclun 100
                       - 0.100 Leaf Type*UV-C Dosage_Mesclun 150
                       + 0.007 Leaf Type*UV-C Dosage_Rocket 0
                       - 0.446 Leaf Type*UV-C Dosage_Rocket 100
                       + 0.439 Leaf Type*UV-C Dosage_Rocket 150
                       - 0.598 Leaf Type*UV-C Dosage_Spinach 0
                       + 0.577 Leaf Type*UV-C Dosage_Spinach 100
                       + 0.022 Leaf Type*UV-C Dosage_Spinach 150
                       - 0.082 Sanitization*UV-C Dosage_No 0
                       + 0.063 Sanitization*UV-C Dosage_No 100
                       + 0.019 Sanitization*UV-C Dosage_No 150
                       + 0.082 Sanitization*UV-C Dosage_Yes 0
                       - 0.063 Sanitization*UV-C Dosage_Yes 100
                       - 0.019 Sanitization*UV-C Dosage_Yes 150
                       - 0.088 Leaf Type*Sanitization*UV-C Dosage_Kale No 0
                       + 0.120 Leaf Type*Sanitization*UV-C Dosage_Kale No 100
                       - 0.032 Leaf Type*Sanitization*UV-C Dosage_Kale No 150
                       + 0.088 Leaf Type*Sanitization*UV-C Dosage_Kale Yes 0
                       - 0.120 Leaf Type*Sanitization*UV-C Dosage_Kale Yes 100
                       + 0.032 Leaf Type*Sanitization*UV-C Dosage_Kale Yes 150
                       + 0.047 Leaf Type*Sanitization*UV-C Dosage_Mesclun No 0
                       - 0.103 Leaf Type*Sanitization*UV-C Dosage_Mesclun No 100
                       + 0.056 Leaf Type*Sanitization*UV-C Dosage_Mesclun No 150
                       - 0.047 Leaf Type*Sanitization*UV-C Dosage_Mesclun Yes 0
                       + 0.103 Leaf Type*Sanitization*UV-C Dosage_Mesclun Yes 100
                       - 0.056 Leaf Type*Sanitization*UV-C Dosage_Mesclun Yes 150
                       + 0.040 Leaf Type*Sanitization*UV-C Dosage_Rocket No 0
                       + 0.123 Leaf Type*Sanitization*UV-C Dosage_Rocket No 100
                       - 0.163 Leaf Type*Sanitization*UV-C Dosage_Rocket No 150
                       - 0.040 Leaf Type*Sanitization*UV-C Dosage_Rocket Yes 0
                       - 0.123 Leaf Type*Sanitization*UV-C Dosage_Rocket Yes 100
                       + 0.163 Leaf Type*Sanitization*UV-C Dosage_Rocket Yes 150
                       + 0.000 Leaf Type*Sanitization*UV-C Dosage_Spinach No 0
                       - 0.140 Leaf Type*Sanitization*UV-C Dosage_Spinach No 100
                       + 0.139 Leaf Type*Sanitization*UV-C Dosage_Spinach No 150
                       - 0.000 Leaf Type*Sanitization*UV-C Dosage_Spinach Yes 0
                       + 0.140 Leaf Type*Sanitization*UV-C Dosage_Spinach Yes 100
                       - 0.139 Leaf Type*Sanitization*UV-C Dosage_Spinach Yes 150
```

Equation averaged over blocks.

Appendix A3: ANOVA of Phase II major factors (leaf type and UV-C dosage)

Factor Information

Factor	Levels	Values
Leaf Type	4	Kale, Mesclun, Rocket, Spinach
Sanitization	2	No, Yes
UV-C Dosage	3	0, 100, 150

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	13	54.0496	4.1577	11.44	0.000
Blocks	1	0.2380	0.2380	0.65	0.424
Linear	6	47.5663	7.9277	21.81	0.000
Leaf Type	3	4.7172	1.5724	4.33	0.011
Sanitization	1	19.1016	19.1016	52.55	0.000
UV-C Dosage	2	23.7475	11.8737	32.67	0.000
2-Way Interactions	6	6.2453	1.0409	2.86	0.023
Leaf Type*UV-C Dosage	6	6.2453	1.0409	2.86	0.023
Error	34	12.3588	0.3635		
Total	47	66.4084			

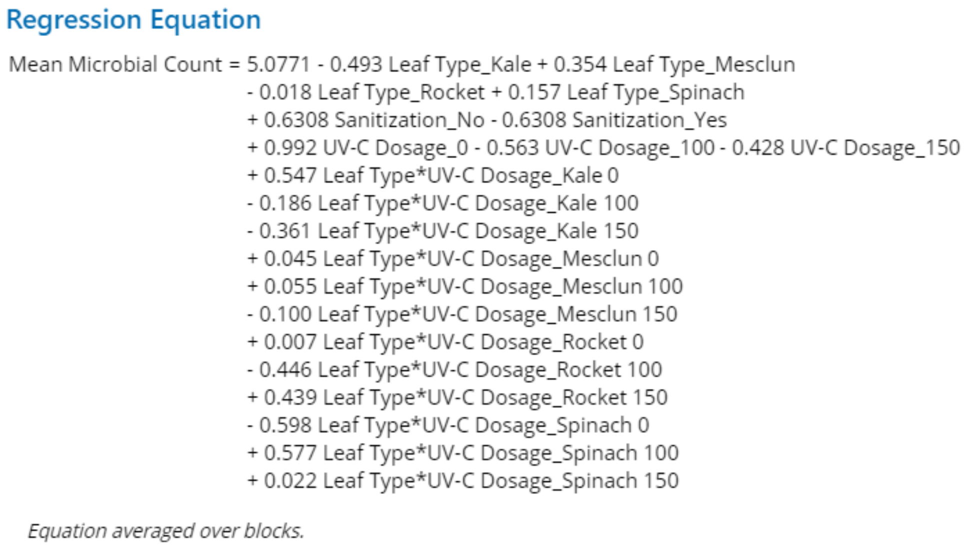
Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.602904	81.39%	74.27%	62.91%

→ Eq. 1

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	5.0771	0.0870	58.34	0.000	
Blocks					
1	-0.0704	0.0870	-0.81	0.424	1.00
Leaf Type					
Kale	-0.493	0.151	-3.27	0.002	1.50
Mesclun	0.354	0.151	2.35	0.025	1.50
Rocket	-0.018	0.151	-0.12	0.906	1.50
Sanitization					
No	0.6308	0.0870	7.25	0.000	1.00
UV-C Dosage					
0	0.992	0.123	8.06	0.000	1.33
100	-0.563	0.123	-4.58	0.000	1.33
Leaf Type*UV-C Dosage					
Kale 0	0.547	0.213	2.56	0.015	2.00
Kale 100	-0.186	0.213	-0.87	0.389	2.00
Mesclun 0	0.045	0.213	0.21	0.834	2.00
Mesclun 100	0.055	0.213	0.26	0.798	2.00
Rocket 0	0.007	0.213	0.03	0.975	2.00
Rocket 100	-0.446	0.213	-2.09	0.044	2.00

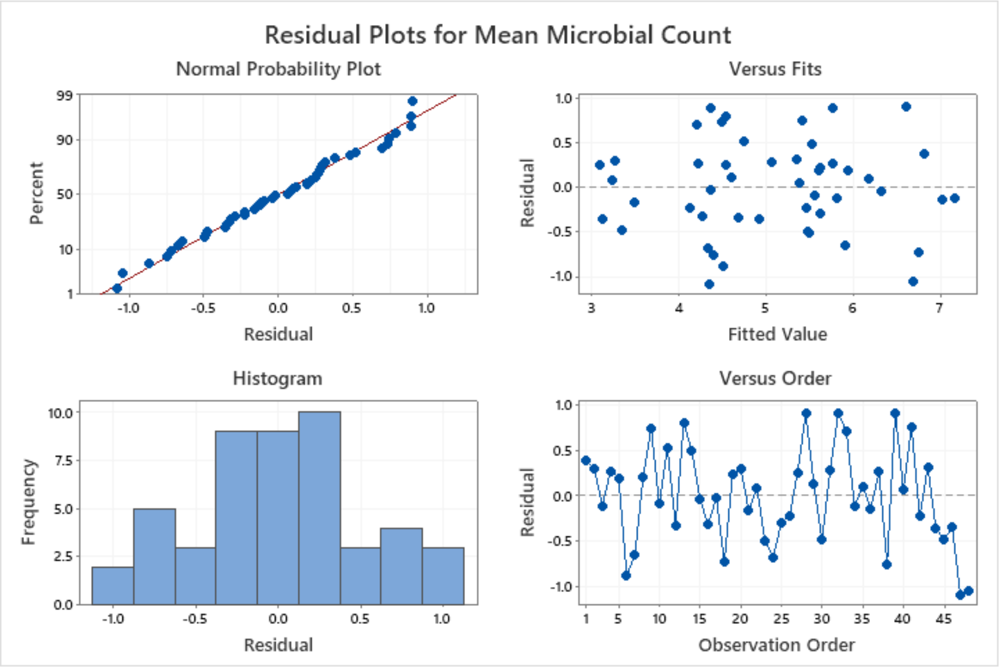


Regression Equation

```
Mean Microbial Count = 5.0771 - 0.493 Leaf Type_Kale + 0.354 Leaf Type_Mesclun
                       - 0.018 Leaf Type_Rocket + 0.157 Leaf Type_Spinach
                       + 0.6308 Sanitization_No - 0.6308 Sanitization_Yes
                       + 0.992 UV-C Dosage_0 - 0.563 UV-C Dosage_100 - 0.428 UV-C Dosage_150
                       + 0.547 Leaf Type*UV-C Dosage_Kale 0
                       - 0.186 Leaf Type*UV-C Dosage_Kale 100
                       - 0.361 Leaf Type*UV-C Dosage_Kale 150
                       + 0.045 Leaf Type*UV-C Dosage_Mesclun 0
                       + 0.055 Leaf Type*UV-C Dosage_Mesclun 100
                       - 0.100 Leaf Type*UV-C Dosage_Mesclun 150
                       + 0.007 Leaf Type*UV-C Dosage_Rocket 0
                       - 0.446 Leaf Type*UV-C Dosage_Rocket 100
                       + 0.439 Leaf Type*UV-C Dosage_Rocket 150
                       - 0.598 Leaf Type*UV-C Dosage_Spinach 0
                       + 0.577 Leaf Type*UV-C Dosage_Spinach 100
                       + 0.022 Leaf Type*UV-C Dosage_Spinach 150
```

Equation averaged over blocks.

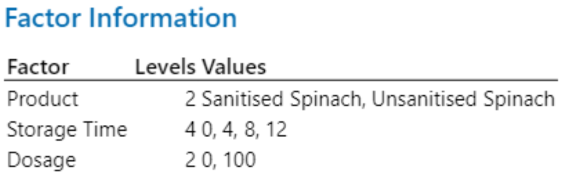
Appendix A4: The four-in-one residual plot for the final model



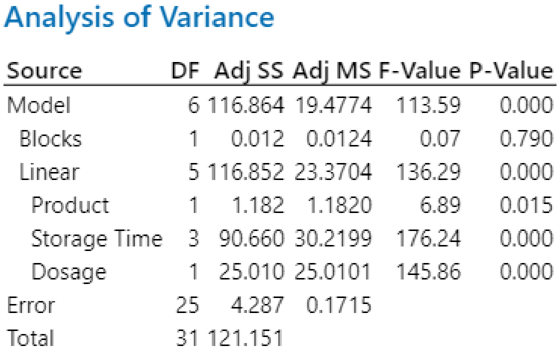
Appendix B1: Microbial Analysis of Spinach for Phase III (Day 0 – 12)

Table B1 Microbial count of UV-C treated and untreated sanitised and unsanitised spinach during storage at 4° C for 12 days

Storage time (day)	Dosage (mJ/cm^2)	Unsanitised Spinach		Sanitised Spinach	
		Initial mean after treatment (log CFU/cm^2)	Reduction (log CFU/cm^2)	Initial mean after treatment (log CFU/cm^2)	Log Reduction (log CFU/cm^2)
0	0	5.36 ± 0.0	1.43	4.94 ± 0.08	1.35
	100	3.9 ± 0.02		3.58 ± 0.11	
4	0	7.61 ± 0.03	3.05	7.06 ± 0.05	2.60
	100	4.55 ± 0.04		4.45 ± 0.01	
8	0	8.91 ± 0.03	1.03	8.42 ± 0.0	1.69
	100	7.88 ± 0.02		6.73 ± 0.07	
12	0	9.35 ± 0.04	1.27	9.24 ± 0.07	1.39
	100	8.08 ± 0.09		7.84 ± 0.01	

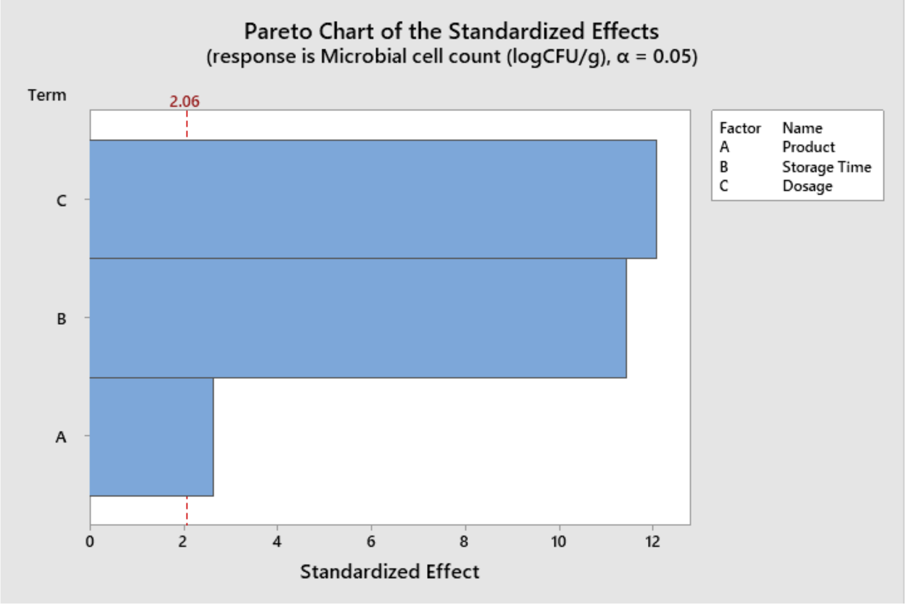


```
Factor Information

Factor        Levels Values
Product            2 Sanitised Spinach, Unsanitised Spinach
Storage Time       4 0, 4, 8, 12
Dosage             2 0, 100
```



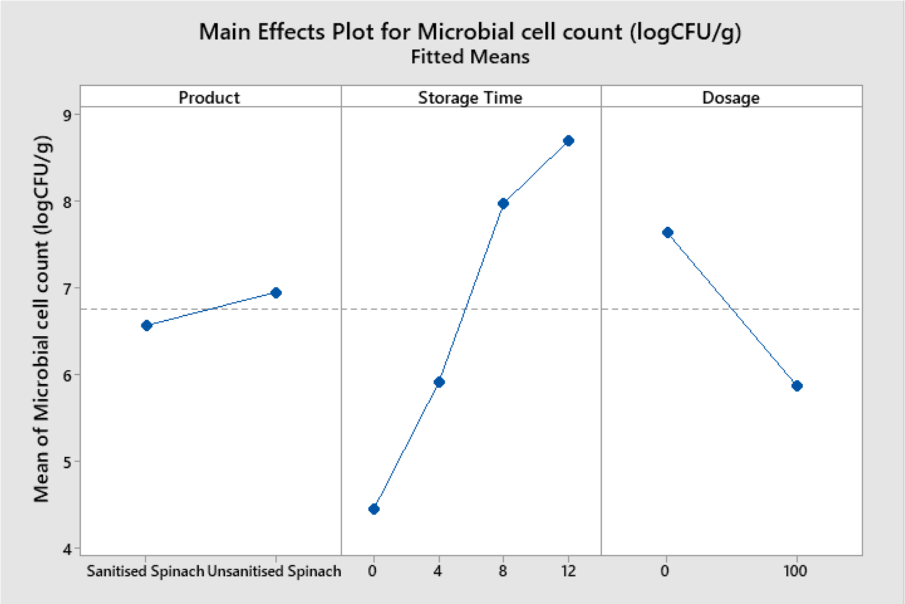
```
Analysis of Variance

Source          DF  Adj SS   Adj MS  F-Value  P-Value
Model            6 116.864  19.4774   113.59    0.000
  Blocks         1   0.012   0.0124     0.07    0.790
  Linear         5 116.852  23.3704   136.29    0.000
    Product      1   1.182   1.1820     6.89    0.015
    Storage Time 3  90.660  30.2199   176.24    0.000
    Dosage       1  25.010  25.0101   145.86    0.000
Error           25   4.287   0.1715
Total           31 121.151
```

Appendix B2: Pareto Chart of standardised effects for microbial cell count (log CFU/cm^2) of spinach



Note: A: Product; B: Storage time; C: UV-C dosage

Appendix B3: Main effect plot for microbial cell count (log CFU/cm^2) of spinach

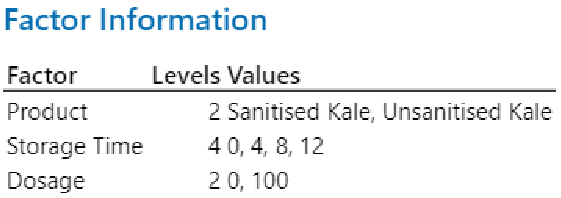


Note: A: Product; B: Storage time; C: UV-C dosage

Appendix B4: Microbial Analysis of Kale (Day 0 – 12)

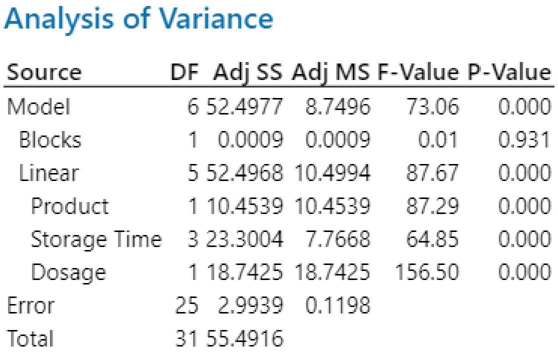
Table B2: Microbial count of UV-C treated and untreated sanitised and unsanitised kale during storage at 4° C for 12 days

Day	**Dosage mJ/cm^2**	**Unsanitised Kale**		**Sanitised Kale**	
		Initial mean after treatment log CFU/cm^2	**Log Reduction log CFU/cm^2**	**Initial mean after treatment log CFU/cm^2**	**Log Reduction log CFU/cm^2**
0	0	6.44 ± 0.09	2.93	4.54 ± 0.00	1.47
	100	3.51 ± 0.04		3.07 ± 0.03	
4	0	6.67 ± 0.01	1.02	5.41 ± 0.04	1.39
	100	5.64 ± 0.03		4.01 ± 0.05	
8	0	7.22 ± 0.04	1.19	6.64 ± 0.07	1.73
	100	6.03 ± 0.03		4.90 ± 0.02	
12	0	7.78 ± 0.07	1.18	6.74 ± 0.04	1.30
	100	6.59 ± 0.09		5.44 ± 0.04	



Factor Information

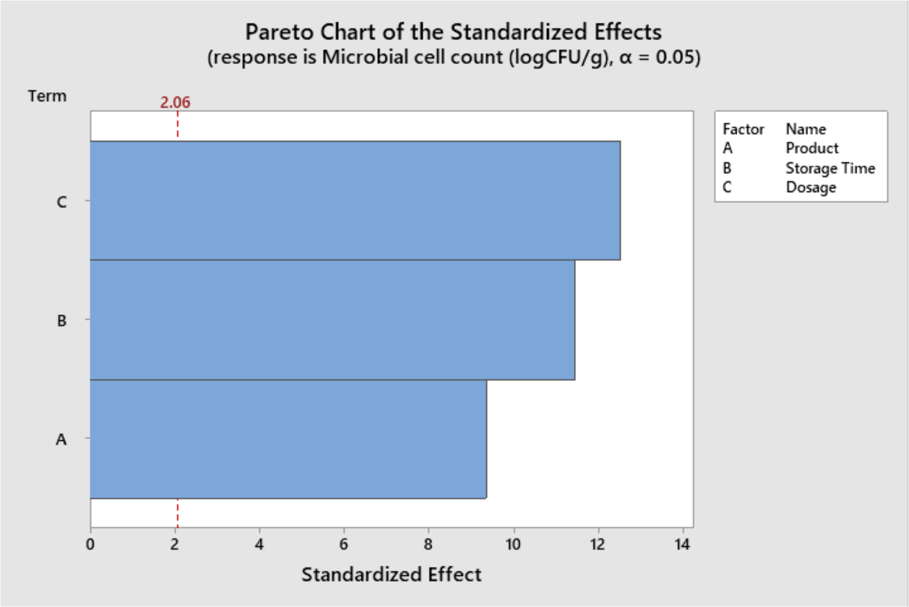
Factor	Levels	Values
Product	2	Sanitised Kale, Unsanitised Kale
Storage Time	4	0, 4, 8, 12
Dosage	2	0, 100



Analysis of Variance

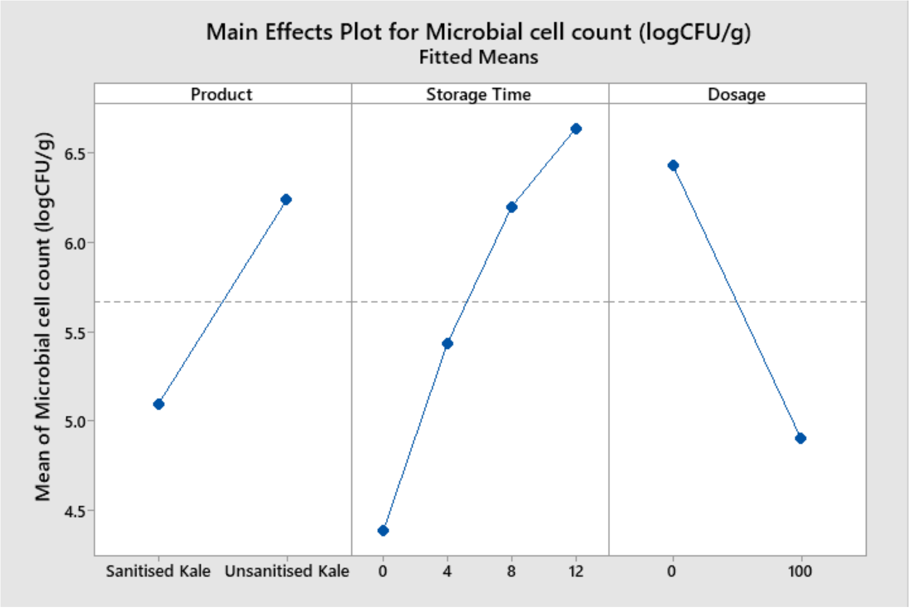
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	6	52.4977	8.7496	73.06	0.000
Blocks	1	0.0009	0.0009	0.01	0.931
Linear	5	52.4968	10.4994	87.67	0.000
Product	1	10.4539	10.4539	87.29	0.000
Storage Time	3	23.3004	7.7668	64.85	0.000
Dosage	1	18.7425	18.7425	156.50	0.000
Error	25	2.9939	0.1198		
Total	31	55.4916			

Appendix B5: Pareto Chart of standardised effects for microbial cell count (log CFU/cm^2) of kale



Note: A: Product; B: Storage time; C: UV-C dosage

Appendix B6: Main effect plot for microbial cell count (log CFU/cm^2) of kale

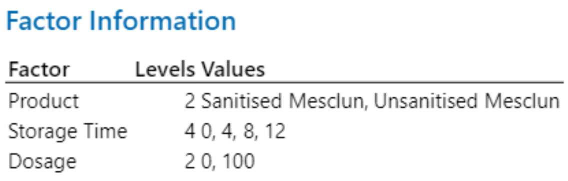


Note: A: Product; B: Storage time; C: UV-C dosage

Appendix B7: Microbial Analysis of Mesclun (Day 0 – 12)

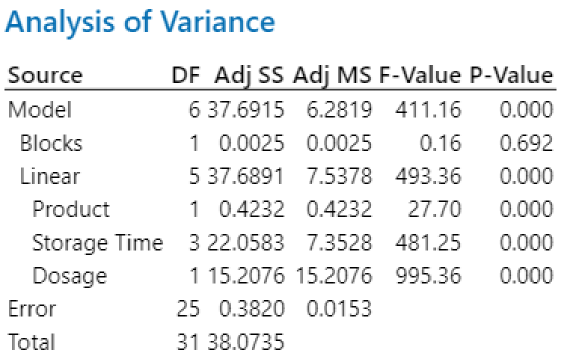
Table B3 Microbial count of UV-C treated and untreated sanitised and unsanitised mesclun during storage at 4° C for 12 days

Day	Dosage mJ/cm^2	Unsanitised Mesclun		Sanitised Mesclun	
		Initial mean after treatment log CFU/cm^2	Log Reduction log CFU/cm^2	Initial mean after treatment log CFU/cm^2	Log Reduction log CFU/cm^2
0	0	7.26 ± 0.23	1.56	7.02 ± 0.08	1.47
	100	5.7 ± 0.00		5.55 ± 0.01	
4	0	8.00 ± 0.00	1.09	7.66 ± 0.04	1.08
	100	6.91 ± 0.01		6.57 ± 0.05	
8	0	9.15 ± 0.07	1.35	9.01 ± 0.01	1.42
	100	7.79 ± 0.02		7.59 ± 0.07	
12	0	9.17 ± 0.02	1.41	9.05 ± 0.03	1.59
	100	7.75 ± 0.05		7.46 ± 0.06	



Factor Information

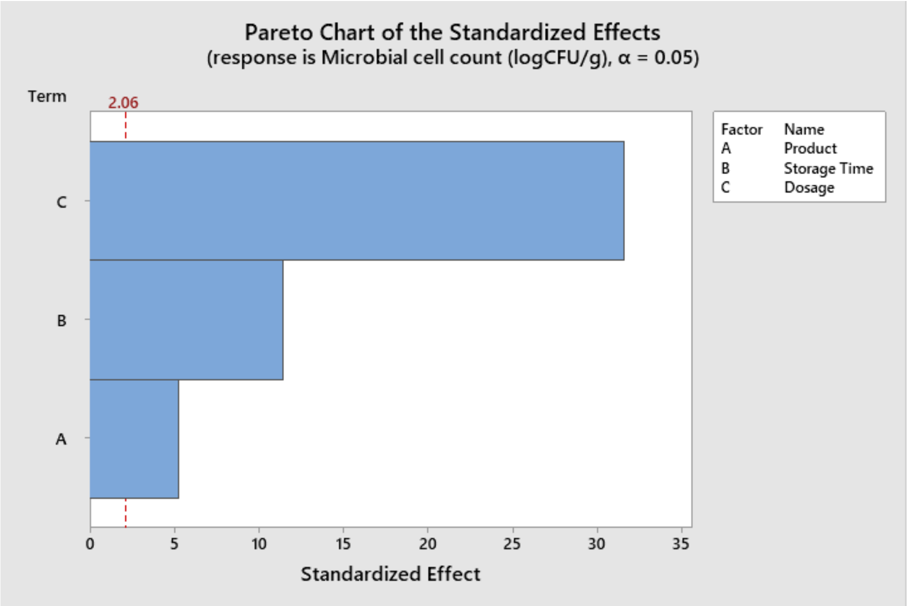
Factor	Levels	Values
Product	2	Sanitised Mesclun, Unsanitised Mesclun
Storage Time	4	0, 4, 8, 12
Dosage	2	0, 100



Analysis of Variance

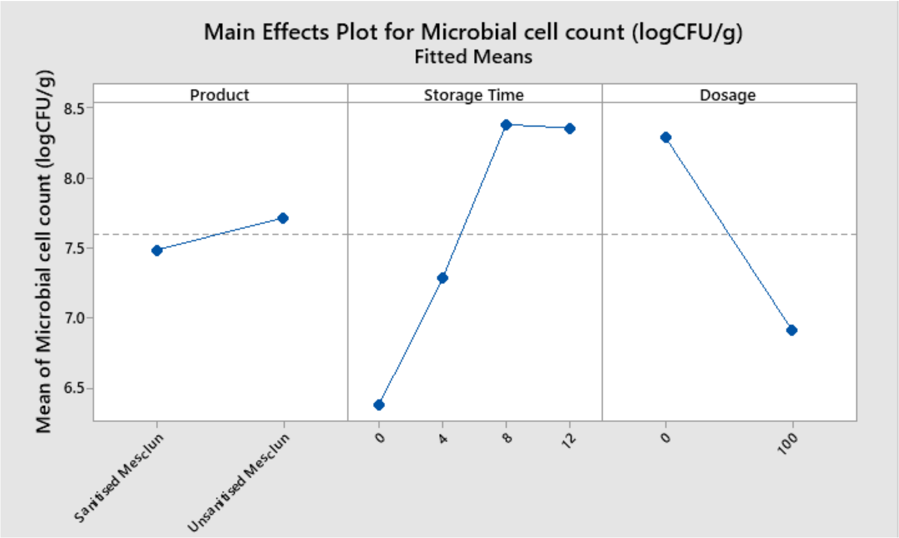
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	6	37.6915	6.2819	411.16	0.000
Blocks	1	0.0025	0.0025	0.16	0.692
Linear	5	37.6891	7.5378	493.36	0.000
Product	1	0.4232	0.4232	27.70	0.000
Storage Time	3	22.0583	7.3528	481.25	0.000
Dosage	1	15.2076	15.2076	995.36	0.000
Error	25	0.3820	0.0153		
Total	31	38.0735			

Appendix B8: Pareto Chart of standardised effects for microbial cell count (log CFUcm^2) of mesclun



Note: A: Product; B: Storage time; C: UV-C dosage

Appendix B9: Main effect plot for microbial cell count (log CFU/cm^2) of mesclun

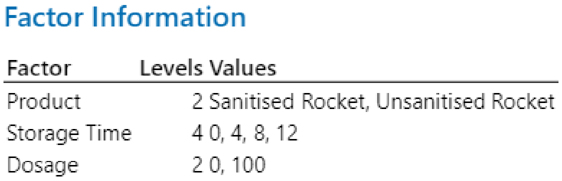


Note: A: Product; B: Storage time; C: UV-C dosage

Appendix B10: Microbial Analysis (Day 0 – 12)

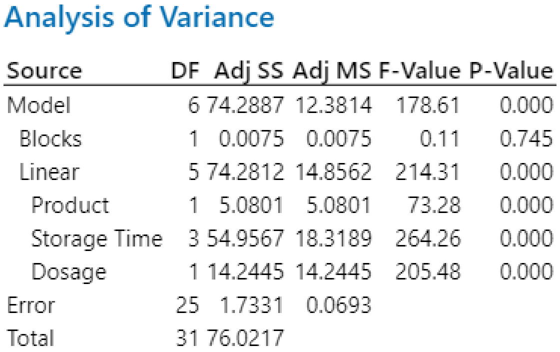
Table B4 Microbial count of UV-C treated and untreated sanitised and unsanitised rocket during storage at 4° C for 12 days

Day	**Dosage mJ/cm^2**	**Unsanitised Rocket**		**Sanitised Rocket**	
		Initial mean after treatment log CFU/cm^2	**Log Reduction log CFU/cm^2**	**Initial mean after treatment log CFU/cm^2**	**Log Reduction log CFU/cm^2**
0	0	6.62 ± 0.02	1.98	5.52 ± 0.1	1.88
	100	4.64 ± 0.07		3.64 ± 0.11	
4	0	8.16 ± 0.00	1.05	7.1 ± 0.02	1.15
	100	7.1 ± 0.03		6.04 ± 0.00	
8	0	8.73 ± 0.03	1.1	7.97 ± 0.00	1.09
	100	7.63 ± 0.02		6.88 ± 0.03	
12	0	9.37 ± 0.00	0.89	9.06 ± 0.02	1.24
	100	8.47 ± 0.09		7.81 ± 0.03	



Factor Information

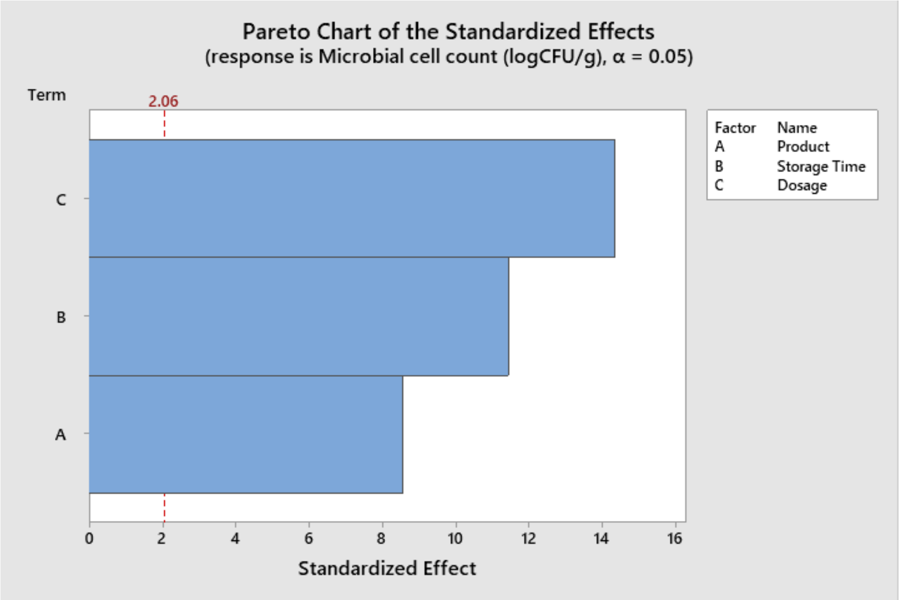
Factor	Levels	Values
Product	2	Sanitised Rocket, Unsanitised Rocket
Storage Time	4	0, 4, 8, 12
Dosage	2	0, 100



Analysis of Variance

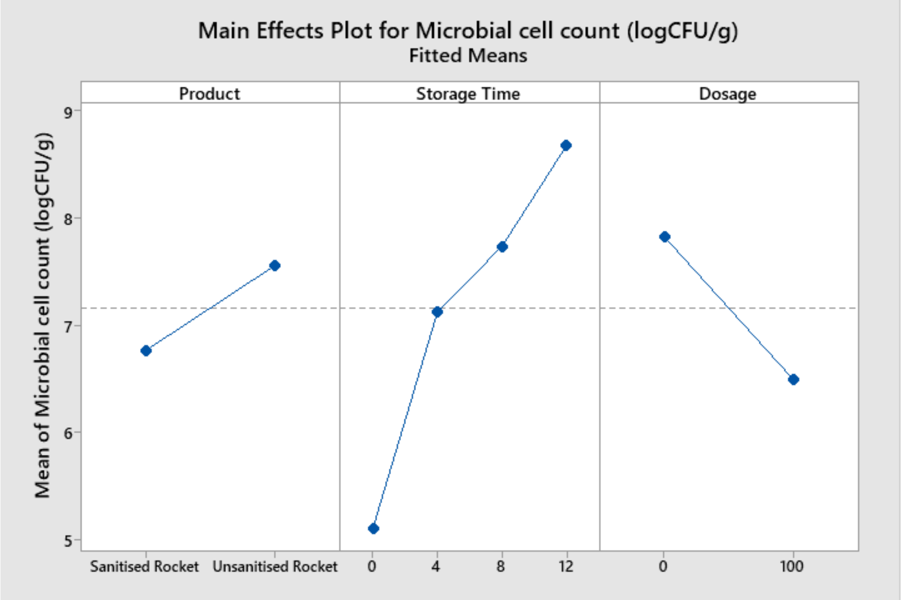
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	6	74.2887	12.3814	178.61	0.000
Blocks	1	0.0075	0.0075	0.11	0.745
Linear	5	74.2812	14.8562	214.31	0.000
Product	1	5.0801	5.0801	73.28	0.000
Storage Time	3	54.9567	18.3189	264.26	0.000
Dosage	1	14.2445	14.2445	205.48	0.000
Error	25	1.7331	0.0693		
Total	31	76.0217			

Appendix B11: Pareto Chart of standardised effects for microbial cell count (log CFU/cm^2) of rocket



Note: A: Product; B: Storage time; C: UV-C dosage

Appendix B12: Main effect plot for microbial cell count (log CFU/cm^2) of rocket



Note: A: Product; B: Storage time; C: UV-C dosage

Appendix C1: Raw data for colour analysis of spinach on day 0

Sample	L*	a*	b*
US-0	98.42	-1.43	2.18
	93.05	-2.31	2.41
	96.25	-1.68	2.18
	96.22	-2.24	2.16
	94.32	-1.95	2.18
US-100	92.05	-2.36	2.22
	96.52	-1.63	2.04
	95.33	-1.36	2.18
	99.6	-2.48	2.23
	91.05	-1.1	2.16
SS-0	109.16	-1.31	2.14
	102.45	-2.17	2.18
	103.75	-2.33	2.14
	105.21	-1.87	2.26
	106.12	-2.1	2.19
SS-100	96.33	-1.73	2.14
	98.52	-1.53	2.15
	94.28	-1.2	2.24
	101.58	-2.54	2.15
	97.86	-2.57	2.27

Appendix C2: Raw data for colour analysis of spinach on day 4

Sample	L	a	b
US0	95.64	-1.5	2.39
	90.23	-1.62	2.25
	96.15	-2.91	2.34
	91.62	-1.32	2.25
	94.5	-2	2.34
US100	95.72	-1.86	2.34
	99.34	-1.23	2.36
	96.65	-1.95	2.24
	96.14	-2.14	2.25
	98.96	-0.92	2.31
SS0	100.41	-1.15	2.29
	101.29	-1.93	2.32
	99.51	-1.85	2.32
	103.2	-2.64	2.39
	100.68	-2.21	2.32
SS100	97.2	-2.49	2.15
	96.42	-1.68	2.31
	97.19	-1.11	2.22
	95.84	-0.96	2.25
	95.9	-1.41	2.13

Appendix C3: Raw data for colour analysis of spinach on day 8

Sample	L	a	b
US0	96.38	-1.35	2.83
	96.25	-1.25	2.65
	94.30	-1.86	2.33
	95.51	-0.95	2.54
	94.84	-1.78	2.78
US100	90.76	-1.02	2.53
	89.42	-1.7	2.51
	90.11	-0.85	2.44
	90.52	-1.62	2.42
	89.6	-0.54	2.51
SS0	99.08	-1.94	2.66
	98.75	-1.68	2.54
	94.62	-0.92	2.48
	90.14	-0.78	2.45
	90.95	-1.68	2.58
SS100	87.7	-1.54	2.02
	88.27	-0.85	2.42
	86.23	-1.56	2.66
	87.15	-0.84	2.18
	86.27	-1.03	2.65

Appendix C4 : Raw data for colour analysis of spinach on day 12

Sample	L	a	b
US0	100.85	-0.26	3.18
	99.14	-0.52	3.17
	98.62	-1.27	2.25
	100.12	-1.68	2.94
	98.61	-1.53	2.86
US100	80.76	-0.24	2.21
	83.12	-0.99	2.36
	80.62	-0.74	2.64
	81.7	-1.02	2.82
	81.35	-1.33	2.65
SS0	96.21	-0.43	3.75
	95.31	-0.55	2.36
	96.28	-1.39	2.23
	94.68	-1.26	3.22
	96.34	-1.37	2.31
SS100	83.94	-1.25	2.78
	85.1	-0.32	2.56
	83.71	-1.04	2.18
	84.53	-1.43	2.62
	84.27	-0.41	2.78

Appendix C5: Raw data for colour analysis of kale on day 0

Sample	L	a	b
UK-0	98.48	-2.56	2.8
	97.53	-1.82	1.54
	98.22	-1.94	2.64
	97.26	-2.67	2.81
	98.77	-1.96	2.37
UK-100	98.08	-2.35	1.84
	97.52	-2.37	2.41
	98.61	-1.52	2.57
	98.38	-2.61	1.48
	98.27	-2.47	2.89
SK0	96.48	-1.96	2.17
	96.17	-2.85	2.28
	95.52	-2.78	2.57
	95.34	-1.92	2.66
	95.87	-1.79	2.61
SK100	98.96	-2.64	2.64
	96.89	-1.73	2.64
	98.72	-1.56	2.51
	97.93	-2.48	1.22
	97.94	-2.46	2.59

Appendix C6: Raw data for colour analysis of kale on day 4

Sample	L	a	b
SK0	96.1	-2.34	2.84
	97.17	-2.46	2.37
	95.26	-1.29	2.98
	96.52	-2.15	2.55
	95.78	-1.48	2.15
UK0	98.74	-1.88	2.64
	97.19	-1.84	2.14
	97.26	-2.12	2.71
	96.42	-1.95	2.63
	95.19	-2.36	2.18
SK100	96.83	-2.12	2.06
	95.29	-2.24	3.26
	94.25	-2.19	3.26
	94.82	-1.96	2.51
	96.22	-1.51	1.95
UK100	95.82	-2.86	2.87
	95.25	-2.34	2.45
	95.78	-1.41	2.68
	97.25	-1.54	2.77
	96.37	-2.08	2.59

Appendix C7: Raw data for colour analysis of kale on day 8

Sample	L	a	b
UK0	96.33	-1.53	2.75
	97.36	-1.42	3.02
	96.59	-2.36	2.51
	96.69	-2.17	3.01
	98.11	-1.19	2.23
UK100	91.04	-2.02	2.81
	93.07	-1.9	2.76
	94.79	-1.48	2.81
	93.95	-1.51	2.92
	94.02	-2.57	3.96
SK0	97.43	-2.7	4.26
	95.91	-1.35	2.5
	94.02	-1.95	2.57
	95.45	-1.26	2.81
	95.48	-1.91	2.37
SK100	94.22	-1.75	2.81
	93.37	-2.55	3.76
	94.62	-1.24	2.81
	94.88	-1.34	2.92
	94.72	-2.1	2.96

Appendix C8: Raw data for colour analysis of kale on day 12

Sample	L	a	b
UK0	96.23	-1.51	2.7
	97.18	-2.09	2.98
	94.13	-1.78	3.13
	95.28	-2.06	4.41
	93	-1.29	2.54
UK100	92.47	-2.31	4.41
	91.56	-2.05	3.7
	93.01	-1.33	1.09
	92.34	-1.68	4.22
	92.08	-1.7	3.6
SK0	95.4	-2.2	2.56
	96.83	-1.91	3.16
	93.33	-1.13	3.29
	96.43	-1.7	3.05
	93.63	-2	4.02
SK100	91.17	-2.86	3.86
	92.72	-2.05	4.12
	93.21	-1.11	3.61
	93.33	-1.29	2.79
	92.33	-1.21	3.24

Appendix C9: Raw data for colour analysis of mesclun on day 0

Sample	L	a	b
UM0	111	-2.41	1.63
	110.99	-2.83	1.93
	82.28	-1.3	1.01
	85.17	-1.49	1.32
	104.58	-1.35	1
UM100	102.21	-1.24	1.47
	92.09	-2.41	1.68
	101.69	-1.83	1.29
	96.63	-2.2	-1.3
	113.69	-1.23	2.3
SM0	91.54	-1.41	1.32
	91.47	-1.16	1.33
	86.54	-2.63	1.65
	111.62	-1.86	1.11
	107.84	-2.96	1.9
SM100	96.21	-1.42	1.82
	106.93	-2.83	1.08
	102.43	-1.21	2.16
	89.35	-1.23	1.68
	85.3	-2	1.42

Appendix C10: Raw data for colour analysis of mesclun on day 4

Sample	L	a	b
UM0	91.43	-1.34	2.68
	100.98	-2.07	1.86
	108.28	-1.43	2.54
	107.93	-1.32	3.31
	94.07	-1.07	2.91
UM100	94.95	-1.04	2.3
	98.14	-1.76	1.15
	92.46	-1.48	2.19
	92.97	-1.44	3.17
	90.27	-1.28	1.33
SM0	96.31	-1.48	1.72
	95.45	-2.31	2.15
	103.99	-2.34	1.31
	100.54	-1.16	2.1
	99.75	-1.15	1.1
SM 100	91.15	-1.86	2.3
	92.89	-0.89	2.58
	95.13	-1.16	3.36
	96.06	-1.33	1.54
	94.41	-1.79	2.81

Appendix C11: Raw data for colour analysis of mesclun on day 8

Sample	L	a	b
UM0	114	-1.23	2.72
	115.57	-0.75	2.48
	97.3	-1.72	3.69
	95.28	-0.83	3.1
	120.61	-1.12	4.09
UM100	84.98	-1.5	3.22
	83.62	-1.82	3.45
	88.62	-0.48	2.38
	98.53	-0.25	2.95
	98.03	-1.61	2.38
SM0	98.07	-1.71	2.08
	98.78	-1.77	3.02
	90.69	-1.55	3.15
	107.68	-0.97	2.26
	85.23	-1.14	2.11
SM100	92.49	-1.17	1.69
	90.02	-1.55	4.15
	93.64	-0.75	3.36
	127.8	-0.72	3.27
	83.58	-1.32	2.97

Appendix C12: Raw data for colour analysis of mesclun on day 12

Sample	L	a	b
UM0	97.78	-0.33	3.76
	83.03	-1.03	4.82
	104.01	-1.17	3.05
	107.86	-0.65	4.04
	83.87	-0.98	3.79
UM100	86.17	-0.86	2.86
	84.72	-1.05	3.12
	90.21	-1.11	3.61
	87.33	-0.29	3.79
	90.33	-1.21	4.24
SM0	104.41	-0.82	3.42
	105.29	-0.59	2.73
	94.27	-1.6	3.06
	96.09	-1.58	2.93
	82.25	-1.42	3.01
SM100	87.47	-0.81	3.41
	88.56	-1.05	3.2
	91.01	-0.73	3.09
	89.34	-0.68	3.22
	88.08	-0.7	3.6

Appendix C13: Raw data for colour analysis of rocket on day 0

Sample	L	a	b
UR0	90.38	-2.37	1.34
	87.34	-2.46	2.47
	89.89	-2.05	1.26
	88.22	-2.57	1.76
	86.57	-2.11	2
UR100	90.98	-2.37	1.34
	88.71	-2.26	2.12
	90.3	-2.48	2.04
	89.18	-2.45	1.59
	90.01	-2.33	1.61
SR0	94.09	-2.93	2.36
	93.36	-2.57	1.23
	93.99	-2.25	1.69
	92.32	-2.26	1.1
	93.53	-2.25	2.37
SR100	88.36	-2.54	1.56
	94.09	-2.24	2.12
	92.32	-2.41	1.65
	86.16	-2.28	1.97
	92.24	-2.75	2.34

Appendix C14: Raw data for colour analysis of rocket on day 4

Sample	L	a	b
UR0	100.44	-2.7	2.93
	100.69	-1.29	2.62
	93.68	-2.01	2.39
	96.34	-1.44	2.86
	96.01	-1.57	1.12
UR100	95.25	-2.64	1.93
	91.9	-1.33	2.79
	97.71	-2.49	2.31
	95.44	-2.51	2.21
	89.83	-1.31	1.97
SR0	95.39	-2.43	1.71
	96.32	-2.84	1.8
	96.2	-2.47	1.75
	95.85	-2.27	2.73
	95.44	-2.26	1.36
SR100	90.91	-2.32	2.97
	92.04	-2.83	2.93
	91.06	-1.2	1.17
	90.43	-2.65	2.76
	90.66	-2.85	1.23

Appendix C15: Raw data for colour analysis of rocket on day 8

Sample	L	a	b
UR0	94.82	-1.83	2.73
	95.31	-2.46	2.87
	104.38	-0.66	3.61
	94.23	-0.87	3.05
	104.78	-1.08	3.92
UR100	97.72	-0.72	3.5
	96.44	-1.02	2.81
	99.3	-1.99	3.04
	98.1	-1.64	3.08
	100.92	-1.89	2.76
SR0	94.42	-2.62	2.48
	93.51	-2.55	3.29
	94.19	-2.59	2.82
	101.07	-1.47	2.54
	94.58	-1.51	3.05
SR100	98.41	-1.33	2.39
	104.14	-2.81	3.04
	98.63	-1.43	2.77
	98.66	-1.72	3.61
	99.6	-1.52	2.74

Appendix C16: Raw data for colour analysis of rocket on day 12

Sample	L	a	b
UR0	99.86	-1.78	3.92
	98.44	-1.9	4.89
	96.94	-0.56	4.34
	103.7	-0.55	2.71
	97.51	-0.23	4.11
UR100	98.38	-1.5	4
	97.25	-0.7	3.83
	102.93	-1.6	3.34
	100.21	-0.4	4.98
	96.24	-1.66	2.82
SR0	96.96	-1.62	1.17
	99.42	-1.85	3.91
	101.08	-1.6	3.08
	105.56	-1.79	2.94
	99.89	-1.52	3.7
SR100	102.4	-1.23	3.43
	103.17	-1.55	4.39
	98.84	-0.88	3.44
	100.64	-1.11	3.28
	101.04	-1.21	3.15

Appendix D1: Raw data for weight of spinach on initial and final day

Sample	Day 0	Day 12
US0	10.43g	10.35g
US100	10.51g	10.31g
SS0	10.71g	10.65g
SS100	10.41g	10.34g

Appendix D2: Raw data for weight of kale on initial and final day

Sample	Day 0	Day 12
UK0	10.23	10.14
SK0	10.8	10.77
UK100	10.32	10.21
SK100	10.6	10.52

Appendix D3: Raw data for weight of mesclun on initial and final day

Sample	Day 0	Day 12
UM0	10.80	8.64
	15.48	14.30
SM0	16.40	15.22
	13.85	12.64
UM100	13.07	12.43
	7.11	6.53
SM-100	6.34	6.03
	8.69	8.19

Appendix D4: Raw data for weight of rocket on initial and final day

Sample	Day 0	Day 12
UR0	11.12	9.73
	10.5	9.1
SR0	10.5	10.28
	10.83	10.61
UR100	8.5	8.21
	7.16	6.89
SR-100	10.42	10.14
	10.1	9.88