Effect of Pulsed Light (PL) and UV-C light treatments on the shelf-life and quality enhancement of fresh carrot and radish

By

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ABSTRACT

A balanced diet is very essential to stay healthy. Fresh fruits and vegetables contribute the most important part of a balanced diet. There are numerous challenges in the supply chain management to ensure that fresh produce can reach the consumer as clean, fresh, wholesome and free of undesirable contaminants as possible. Pulsed light (PL) treatment is a promising technology which can be used on fresh produce to enhance some quality enhancement. As a defense response mechanism against the harsh/unfavorable treatment, the treated fruit or vegetable produces secondary metabolites such as antioxidants and total phenolic compounds. The first objective of the study focused on PL treatment of fresh carrots (both cut and whole) for quality and shelf life enhancement. The ultraviolet light treatment (UV-C), which is known in industry for its germicidal properties, has been used for environmental air and drinking water decontamination. The second objective was focused on a comparative evaluation of PL versus UV-C light treatment on quality changes and shelf life of another vegetable, red radish.

Only minimal changes in the overall appearance during the shelf life of PL treated whole carrot were observed as compared to PL treated cut carrots (*Daucus carota*) and total microflora in test samples also was very minimally affected demonstrating only a small reduction in surviving microflora (0.12-0.42 logarithmic cycle reduction). The study demonstrated that PL treatment did not significantly increase the overall shelf life of carrots. However, it demonstrated the significantly positive impact on quality enhancement. Peroxidase enzyme retained its activity in both (cut and whole carrots), while the antioxidant activity showed a large increase, by several folds, along with the higher log reduction was achieved for aerobic and yeast & mold count. PL treatment resulted in lowering of changes in pH for whole carrots possibly by microbial control.

In the case of red radish (*Raphanus sativus*), the overall appearance of treated radish was found to change during the study which again showed only a small reduction in total microflora (0.12-1.07 logarithmic cycles). The skin color of untreated and treated radish turned from "shining red" to "dark black" during storage at room temperature, and the pulsed light and UV-C treatments at the highest dosage was responsible for faster rate of deterioration. However, the antioxidant activity of light treated samples increased during storage up to several folds, when compared with the untreated samples.

Further research is needed to understand the adverse effects on specific commodity which could lead to the design and commercial application of this technology.

RÉSUMÉ

Un régime alimentaire équilibré essentielle pour rester en bonne santé. Les fruits et légumes frais constituent la partie la plus importante d'un régime alimentaire équilibré. Il existe de nombreux défis dans la gestion de la chaîne d'approvisionnement pour garantir que les produits frais peuvent arriver au consommateur aussi propre, frais, sain et exempt de contaminants indésirables que possible. Le traitement à la lumière pulsée (LP) est une technologie prometteuse qui peut être utilisée sur les produits frais pour augmenter certaines améliorations de la qualité. En tant que mécanisme de réponse de défense contre le traitement dur / défavorable, il produit des métabolites secondaires tels que des antioxydants et des composés phénoliques totaux. Le traitement à la lumière ultraviolette (UV-C), qui est connu dans l'industrie pour ses propriétés germicides, a été utilisé pour la décontamination de l'air et de l'eau potable dans l'environnement. Cela a dérivé le premier objectif comme une étude du traitement PL sur les changements de qualité de carottes fraîches (coupées et entières) et l'amélioration de la durée de conservation. En outre, la recherche s'est étendue au deuxième objectif en tant qu'étude comparative du traitement de la lumière PL par rapport aux rayons UV-C sur les changements de qualité et la durée de conservation de l'ensemble du radis.

Seuls des changements minimes de l'apparence globale au cours de la durée de conservation des carottes entières traitées au LP ont été observés par rapport aux carottes coupées traitées au LP (*Daucus carota*) et la microflore totale dans les échantillons d'essai a également été très peu affectée, démontrant seulement une petite réduction de la microflore survivante (réduction du cycle logarithmique de 0,12 à 0,42). L'étude a démontré que le traitement au LP n'augmentait pas de manière significative la durée de conservation globale des carottes. Cependant, il a démontré l'impact significativement positif sur les paramètres de qualité. Le traitement au LP a entraîné des changements de pH plus faibles pour les carottes entières. En outre, l'enzyme peroxydase n'a pas été désactivée pour les deux (carottes coupées et entières), tandis que l'activité antioxydante a montré une augmentation de plusieurs fois, ainsi qu'une réduction logarithmique plus élevée a été obtenue pour le nombre d'aérobies et de levures et moisissures.

Dans le cas du radis (*Raphanus sativus*), l'apparence globale du radis traité a changé au cours de l'étude, mais encore la microflore totale ne montrant qu'une faible réduction (0,12-1,07 cycle logarithmique). La couleur de la peau des radis non traités et traités est passée du rouge brillant au noir foncé pendant le stockage à température ambiante, et les traitements à la lumière

(LP et UV-C) à la dose plus élevée étaient responsables d'une accélération du taux de détérioration dans le cas des radis. Cependant, l'activité antioxydante des échantillons traités à la lumière a augmenté pendant le stockage jusqu'à plusieurs fois, par rapport aux échantillons non traités.

Des recherches supplémentaires sont nécessaires pour comprendre les effets néfastes sur des produits spécifiques et qui pourraient conduire à la conception et à l'application commerciale de cette technologie.

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CONTRIBUTION OF AUTHORS

This thesis has been written in manuscript style to suitably edit chapters and highlighting research for publication. A part of this thesis research has been presented in CTAQ meeting and the results have also been communicated to the CTAQ partners during the several meetings held in 2017 and 2018.

Authors involved in the thesis work and their contributions to the various articles are as follows:

Prasant Prusty is the MSc. candidate who planned and conducted all the experiments, on the advice and guidance of his supervisor, gathered and analyzed the results and prepared drafts for the thesis and the manuscripts for scientific presentations and publications.

Dr. Hosahalli S. Ramaswamy is the thesis supervisor, under whose guidance the research was completed. He coordinated and supervised the candidate in planning and conducting the research, as well as in correcting, reviewing and editing the thesis.

ABBREVIATIONS

PL	Pulsed light
PLT	Pulsed light technology
UV	Ultraviolet light
E	Energy emitted in the form of light
h	Planck's constant (6.23×10^{-34} J x s)
c	Speed of the light, m/s
λ	Wavelength of the radiation, m
I_o	Initial energy
I	Actual intensity of light available in the food matrix
T	Transparency coefficient of the food material
x	The distance travelled by the light in the food matrix
W	Watt
mW/cm^2	Milliwatt per square centimeter
WHO	World Health Organization
FAO	Food and agriculture organization
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
CFR	Code of federal regulations
m	Meter
s	Second
ns	Nanosecond
ms	Millisecond
J/cm^2	Joules/square centimeter
nm	Nanometer
λ	Lamba, wavelength
˚C	Degree celsius
kJ/m^2	Kilojoules/square meter
cfu/g	Colony forming units per gram
%	Percent
ES	Esterase activity

CHAPTER 1

INTRODUCTION

A balanced diet is essential to stay healthy. Fresh fruits and vegetables are important contributors to any balanced diet. According to the report published in 2004 by World Health Organization / Food and Agriculture Organization (WHO/FAO), a minimum intake of 400 g of fruits and vegetables per day can help prevent from major diseases such as cardiovascular diseases, diabetes, obesity and certain types of cancer.

There are numerous challenges in the food supply chain to ensure that the fresh produce reaches the consumer fresh, clean and free of contaminants. Fresh produces are grown in the field and the growing practices can contribute to the wholesomeness of the produce commodity as well as can result in contaminating it. The biggest challenge to any fresh fruit or vegetable produced is that they are highly prone to spoilage and have a relatively short shelf life at room temperature storage (Hammond et al., 2015).

Spoilage of fresh produce can be caused from different sources that may be based on physiological, chemical, microbiological, biological or a combination of all these. Physiological and physical factors that can damage the surface, as well as internal tissues which can accelerate the spoilage activity caused by other agents such as microorganisms. Chemical and biochemical factors that are involved in chemical and enzymes related activities result in quality change such as browning, oxidation and other reactions leading to product spoilage. Microbiological spoilage is the most common types of spoilage in fresh produce and occurs due to action of spoilage bacteria and fungi. Biological spoilage is spoilage due to biological factors such as insect damage which mostly happens during the growing stages.

Different surface treatments are being adopted while processing fresh produce to improve the quality and food safety challenges. It is generally understood and broadly accepted that the microbial contamination by pathogenic and spoilage bacteria generally starts at the produce surface. Again, in most situation the protective tissues of the fruits and vegetables resist the invasion of these microorganisms unless they find avenues for their entry into the fruit or vegetable tissue. Hence many treatments are focused on which include both physical and chemical agents to tackle the microbial contaminants at the produce surface. It is very important for any surface treatment technology to make sure the effect of their application be very gentle

on the surface of the fresh produce so that it does not become a source of any additional damage or safety risk. It is also essential that the surface treatment does not leave any residues, or the residual limit does not add any health and safety risk to the consumers.

The very basic method is washing of fresh produce in clean water which takes away some of the surface contaminants. Washing with hot water or water containing disinfectant is used most widely to reduce the pathogenic and spoilage microorganisms from the surface by two to three logarithmic cycle reductions (Smith et al., 2003). Reduction in the spoilage organisms helps in achieving a longer shelf life.

The negative side of the chemical surface treatment is that there may be chemical residues retained on the surface which may be a health risk. With growing consumer awareness on the nature of these chemicals, the negative public reaction over use of these chemicals in fresh produce to extend shelf life is also growing. Today's consumers are moving away from the traditional eating habits and focusing on all natural, free of chemicals or free of processing aids.

Pulsed light (PL) and ultra-violet (UV) light application as surface treatment on fresh produce represent emerging technologies which are getting a lot of attention from researchers and consumers around the world. The PL technology provides a very high instantaneous energy in extremely short cycles (milliseconds) as compared to the traditional UV light which is given in hours with a low intensity UV light treatment process. UV is known for its germicidal effect and its application on water sanitation, air decontamination etc. have been well commercialized.

The current research was carried out with the following objectives:

1) To study the effect of PL treatment on overall quality and shelf life in fresh whole and cut carrots at different levels of treatments and storage conditions.
2) To evaluate PL and UV-C light treatments with another vegetable (whole fresh radish) with the objective of comparing the effectiveness of UV-C and PL treatments on shelf-life extension and quality preservation.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION: UV-C vs Pulsed Light

From a technological standpoint on the source of UV light there are two different technologies as below:

1) UV-C light technology: This is the traditional method of generating UV-C light from the UV bandwidth of the light (100-400 nm). The source of light is usually mercury lamps and the energy level are very mild. The applied energy is calculated with the power of the lamp with the exposure time. Low cost is the primary benefit of this technology. The challenges with this traditional technology are the hazardous nature of mercury from the lamp, warm-up time and low energy output (hence require longer exposure) for efficient germicidal effect (Crook et al., 2015).
2) Pulsed light technology: PL refers to a broader spectrum of high intense (0.01 to 50 J/cm^2) wavelengths (100-1000nm) applied with pulses for a shorter duration (0.1s to 1µs). The primary source of energy comes from the UV spectrum. The output energy can be controlled through the controller for effective log reduction. Because of this high energy level, this technology is more effective for germicidal application on surfaces. The source of light generation is xenon lamps. The installation cost is very high for PL technology as compared to the UV-C technology (Rowan N., 2019).

2.2 ULTRA VIOLET (UV) LIGHT

An atom consists of a positively charged nucleus consisting of protons and neutrons and rapidly moving negatively charged electrons orbiting the nucleus as illustrated in Figure 2.1. Each orbit has its own unique energy state with orbits close to the nucleus having lower energy. When an orbiting electron makes a transition from a higher energy to a lower energy orbit, energy is emitted in the form of light (OpenStax, 2019).

The amount of energy generated can be explained by using Planck's equation (Eq) 2.1 as below:

$$E = E_2 - E_1 = hc / \lambda \qquad \text{- - - - - -} \text{ (Eq 2.1)}$$

where, E = Energy emitted in the form of light, J

E_2= higher energy state, J

E_1 = Lower energy state, J

h = Planck's constant (6.23 x 10^{-34} J x s),

c = Speed of the light, (m/s) and

λ = wavelength of the radiation, (m).

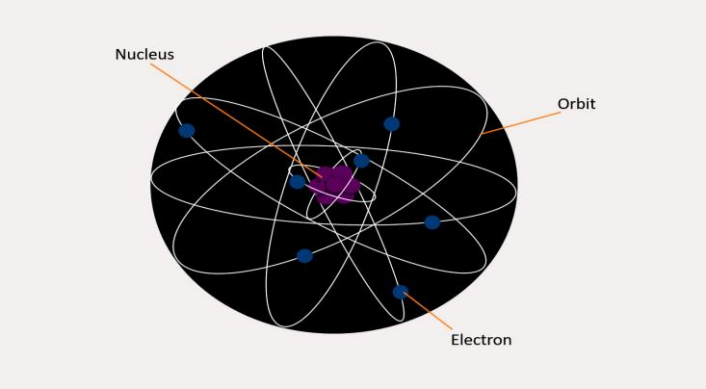


Figure 2.1 Mechanism of UV light generation
(Source: Koutchma et al., 2009)

Ultraviolet (UV) light is the part of the electromagnetic radiation with the wavelength of 100-400 nm (Figure 2.2). UV light is not visible and is just one portion of different electromagnetic radiations. The UV spectrum is again divided in three different parts UV-A, UV-B and UV-C. UV-A falls between the wavelengths 315-400 nm, UV-B between 280-315 nm and UV-C between 200-280 nm. The zone of 100-200 nm is called the vacuum range (Masschelein and Rice, 2002). UV-C portion has the maximum germicidal characteristics and is lethal to microorganisms. This is the effective portion of the UV spectrum from antimicrobial activity point of view. However, the germicidal effect varies depending on the types of microorganism and the exposure treatments (Sharma, 2005). UV light is present in the sunlight and the UV-C spectrum gets filtered through the ozone layer and hence does not make its way to the earth surface.

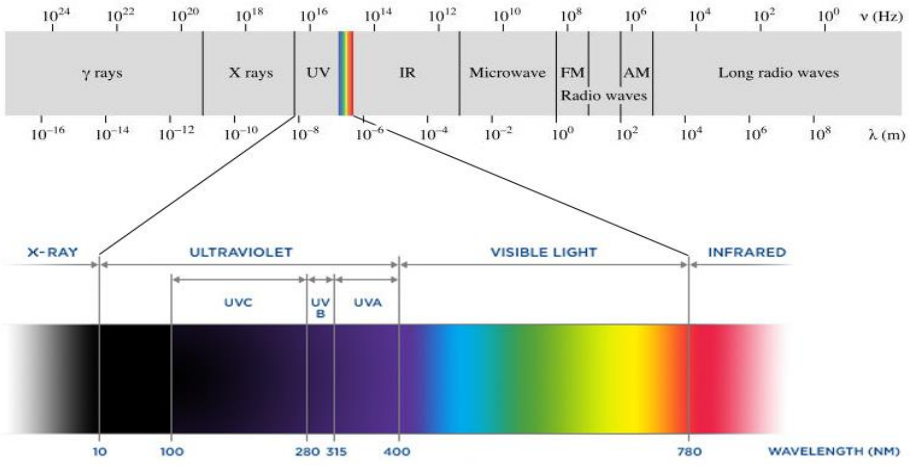


Figure 2.2 Electromagnetic radiation spectrum

(Source: http://insanitek.net/wp-content/uploads/2015/05/Spectrum-publish.jpg)

2.3 MECHANISM AND GERMICIDAL EFFECT OF UV LIGHT

As UV light propagates away from the source of generation, the light interacts with materials it encounters on its way through absorption, reflection, refraction, and scattering processes (Figure 2.3). When the light is absorbed it is no longer available for inactivating microorganism unless interacting with sensitive moieties like DNA. This absorbed energy will result in temperature rise in the receiving body. However, light in reflection, refraction and scattering mode is available for action against microorganisms. Reflection is defined as the change of direction of light deflected from an interface. Scattering is the process of deflection of electromagnetic radiations from a straight path through an absorber (Keener and Krishnamurthy 2014).

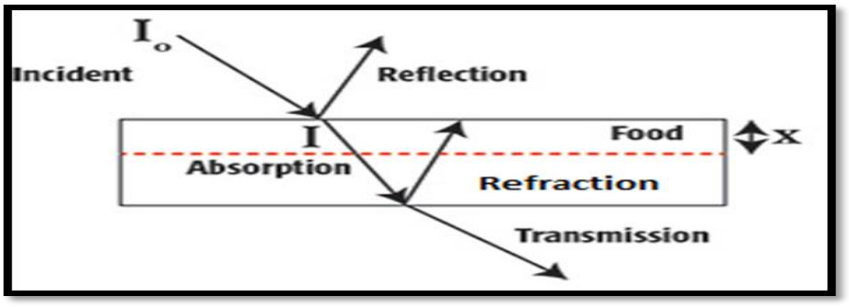


Figure 2.3 Interaction of light and food

[Source: http://www.foodsafetymagazine.com/magazine-archive1/junejuly-2014/shedding-light-on-food-safety-applications-of-pulsed-light-processing

(Keener and Krishnamurthy 2014)]

The intensity of light decreases as it enters the food matrix. The amount of radiated energy at any point in the food matrix can be determined by the Beer-Lambert Law as below equation 2.2 (Swinehart, 1962):

$$I = T I_o e^{-x} \qquad \text{- - - - - - (Eq 2.2)}$$

Where, I_o = the initial energy,

I = actual intensity of light available in the food matrix,

T = transparency coefficient of the food material, and

x = the distance travelled by the light in the food matrix.

Keener and Krishnamurthy (2014) explained, the transparency coefficient is very low in solid foods, and hence the penetration depth could vary from one to few milliliters. Much deeper penetration can be achieved with liquid foods of higher transparency coefficient such as water, beer, juice etc. The mechanism of UV light action depends highly on the actual energy absorbed by the food upon exposure. Also, other factors such as exposure time, energy level, absorption surface are equally important with any UV light surface treatments.

The C-band spectrum of UV light is known for its germicidal effect on microorganisms and considered the strongest portion of the UV radiation (Kowalski, 2009). Wavelength of 253.7 nm is the most powerful wavelength when targeting the nucleic acid of microorganisms (Mara and Horan, 2003). According to Dai et al. (1987), electromagnetic radiation from 240-280 nm effectively damages the nucleic acid and hence inactivates the microorganism. The nucleic acid of any cell is composed of either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). The DNA of most cells carries a double strand. DNA is responsible for the metabolism process in the cell Harris et al. (1987).

Upon UV-C exposure, the nucleic acid absorbs the photon emitted with the radiation. This energy is used in three different ways to create germicidal effect of the microorganisms.

1) DNA Damage: High energy power photon induces the formation of pyrimidine dimers by altering the DNA binding (Figure 2.4). This alternation in the DNA bonding either stops the replication of nucleic acid or inhibits the growth and eventually leads to death.
2) Photo-thermal damage: The radiated energy will overheat the cell contents and cell walls and hence the cell dies.
3) Photo-Physical damage: High radiated energy breaks the cell contents and cell walls and cause cell death.

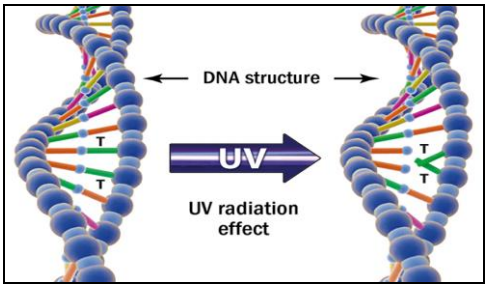


Figure 2.4 Mechanism of UV disinfection

(Source: http://www.ultraviolete.com/about-us)

The success rate of germicidal effect greatly depends on the exposure treatment (wavelength, energy level, source of energy and time), type of microorganism and the surface application (type of food, pH, total solids, suspended solids, absorbance etc.). The dose of UV light required to reduce microbial population by a single decimal order of magnitude referred as the "D_{10} dose," The D_{10} does of various microbes can be found in the Table 2.1 Vegetative bacteria is found easier to kill with UV light than spores and viruses.

Table 2.1 D10 UV Inactivation Doses (mJ/cm^2) Measured at 253.7 nm for Various Microbial Groups (Koutchma et al., 2009)

Microbial group	D_{10} UV dose (mJ/cm^2)
Enteral bacteria	2-8
Cocci and micrococci	1.5-20
Spore formers	4-30
Enteric viruses	5-30
Yeast	2.3-8
Fungi	30-300
Protozoa	60-120
Algae	300-600

Hazen and Sawyer (1992) described the photo-reactivation process, which is a microorganism's ability to repair the damaged DNA under catalyzing effects (i.e. visible wavelengths). The extent of reactivation depends on the organism and the visible wavelength.

2.4 ABIOTIC STRESS MECHANISM BY UV LIGHT

Plant cell metabolites are compounds synthesized by the plant cell for various functions. They are divided into two types such as primary metabolites and secondary metabolites. Primary metabolites (carbohydrates, lipids, protein and nucleic acids) are produced for essential functions such as growth and developments (Dewick, 2001). Secondary metabolites (alkaloids, terpenoids and phenolic compounds) are produced specific functions such as defense against stress. Plant cell produce metabolites for essential functions such as growth (Rabha and Jha, 2018).

Stress can be explained as an environmental factor with potential to create unfavourable condition in living cells. Abiotic stress is defined as the impact of stress from non-living environmental sources to a living organism. Abiotic stresses include temperature, water, light, sound and salinity etc. The living cell adjusted to any abiotic stress condition by producing defense mechanism. The output of the defense mechanism can be temporary until the unfavorable condition is gone or can be permanent within the cell (Isah, 2019).

UV light stress can induce abiotic stress in the plant cells and the stress can stimulate the synthesis of various secondary metabolites such as phenolic compounds (Figure 2.5). Some of these phenolic compounds are found to be retained at some level along with storage post treatment (Murugesan et al., 2012).

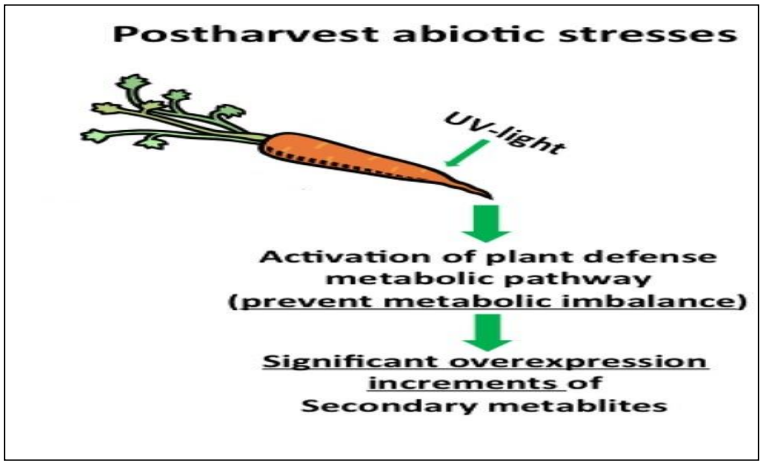


Figure 2.5: Postharvest abiotic stress

(Adapted from Jacobo-Velázquez et al. 2012, Source: http://www.mdpi.com)

2.5 Regulatory compliance with UV application in food

UV light technology is commonly used in industry for water sanitation, environmental air decontamination and food contact surfaces by using the germicidal property of the UV-C wavelength. The positive side of using UV light is that this treatment is known to be inexpensive, needs minimal processing, does not leave any residues in food, known for its germicidal effect and found to enhance quality attributes (Costa et al., 2006).

On the other side of the UV light application, the treatment has to be suitable for the specific application. Severe treatment can cause unfavorable tissue disruption, membrane breakdown and undesirable discoloration. Most kinetic data on microbial inactivation are obtained in aqueous media or air and proven to be effective on smooth surfaces. Fresh produce do not always have uniform surface and can alter the disinfection rate significantly. Consumer awareness on the application of UV light is very limited as on date. With limited consumer awareness, consumer acceptance to this novel technology can be challenging. Despite the above limitation, research on the application of UV light on solid foods for surface decontamination is growing due to the promising outcomes of the technology.

In United States, the U.S. Food and Drug Administration (FDA) has approved the use of ultraviolet radiation for processing and treatment of food under the following guidelines in Code of Federal Regulations Title 21 - FOOD AND DRUGS under Sec. 179.39 (21CFR179.39).

1) Source is mercury lamp emitting at wavelength 253.7 nm
2) Intensity of radiation, 1 W (of 2,537 A. radiation) per 5 to 10 ft^2
3) Radiation must be given in a vacuum or inert atmosphere

In Canada, Health Canada has defined guidelines for the application of Ultraviolet light under the "Novel Process" with proper safety assessment. The Department of Novel Foods of Health Canada conducted a comprehensive risk assessment for UV-treated apple juice/cider. As part of this assessment, effectiveness of CiderSure 3500 UV-light unit is assessed in reducing the bacterial load in apple/cider juice. Potential adverse chemical and toxicological concerns were also evaluated as part of the assessment. It was concluded that the UV-light treatment can contribute to significant reduction in the microbial load and there is no food safety risk associated with unpasteurized, CiderSure 3500 UV-light treated apple/cider juice (Health Canada, 2004). However, it was also noted that depending on the original microbial load, the post treated microbial load can vary. Reduction does not mean complete elimination of microbial organism and manufacturers requires to take additional control in their production process to

limit the risk of contaminations. Importantly, Health Canada needs to be notified when the process causes the food to undergo a major change.

Arvanitoyannis (2010) mentioned that ultraviolet treatment is considered as a part of irradiation as per the European Union Food Safety Regulation. There are lot of discussion is ongoing on the scope and application of irradiation on specific foods. Although process and guidelines for irradiation have not been harmonized in the European Union, however, based on the current irradiation guidelines, it is authorized with the following requirements:

1) The need for technological aspects
2) Irradiation must not contribute to any health hazard in the food
3) The consumer would be benefited with its use
4) Not used as a substitute for the basic hygiene, manufacturing and agricultural practices

2.6 PULSE LIGHT TECHNOLOGY (PLT)

PLT is a relatively new method of food preservation that was introduced by Pure Pulse Technologies in San Diego California in the 1990s (Pollock, 2007). This technique has received several names in the scientific literature: pulsed UV light, high intensity broad-spectrum pulsed light, pulsed light and pulsed white light (Gomez-Lopez et al., 2007).

UV light can be applied in two modes namely continuous mode and pulsed mode. In continuous mode, UV-light energy is released continuously in a monochromatic or polychromatic wavelength, whereas in the pulsed mode, the electrical energy is stored in a capacitor over a short period of time and released as very short duration pulses (Krishnamurthy and Demirci, 2008). PLT involves the usage of inert-gas flash lamps that convert short duration and high-power electric pulses into short duration, high power pulses of radiation within the frequency regions of UV, visible (VL), and infrared (IR) light (Cacace and Palmieri, 2014). Dunn et al. (1995) described “PL is produced using engineering technologies that multiply power several fold. Power is magnified by accumulating electrical energy in an energy storage capacitor over relatively long times (fractions of a second) and releasing this stored energy to do work in much shorter times (millionths or thousandths of a second). The result is very high power during the duty cycle with the expenditure of only modest average power consumption.”

Various foods which are opaque with complex surfaces, cannot be sterilized by exposure to pulsed light; however pulsed light is very effective for reducing microbial loads on the surface and extending the shelf life of such products (Pollock, 2007). The various study results revealed

that pulsed UV has a higher penetration depth and are more effective than continuous UV light for some studies (Krishnamurthy, 2006; Jun et al., 2003).

Pollock (2007) mentioned a potential for commercial PLT application not only to increase shelf-life but to ensure the microbial safety of the final product. Germicidal properties of pulsed light can almost be attributed to the UV part (UV-C region, 200-280 nm) of the spectrum. The 250-260 nm range is considered to be capable of destroying most microorganisms due to the alteration of DNA (Bintsis et al., 2000). Pulsed light inactivates microorganisms by a combination of photothermal and photochemical methods (Ortega-Rivas, 2012).

Dunn et al. (1997) stated PLT as an economical process as it can treat products on-line at high throughput (approximately 50 packages per minute). This is due to the fact that a single lamp can produce multiple flashes per second and only a few are required to be effective in reducing microbial populations. An in-depth look at study result supported as PLT at 4 J/cm^2 showed usage cost estimates of approximately 1-4 cents/m^2 of treated surface area (including amortization of the capital expenditure, lamp replacement, maintenance, and electrical costs).

Further, this technology could be extended as an alternative to thermal treatment for killing pathogenic and spoilage microorganisms in foods, including bacteria, yeasts, molds, and viruses. Apart from that, PLT could be applied to sterilize or partially decontaminate microbial loads on the surface of packaging materials, transparent pharmaceutical products, surfaces and transmissivity materials, including water, air, and many solutions is well-published (Dunn et al., 1997). Moreover, several PLT systems for different purposes, including food related ones, are produced by a range of commercial companies, including SteriBeam® in Germany, Xenon® Corporation in the United Sates and Claranor® in France (Cacace and Palmieri, 2014).

2.7 Regulatory compliance with PL application in food

The PL method has been approved by the U.S. Food and Drug Administration (FDA) after being evaluated for both safety and effectiveness. FDA has approved the use of PL for the treatment of food under the following guidelines in Code of Federal Regulations Title 21 - FOOD AND DRUGS under Sec. 179.39 (21 CFR 179.41) for surface microorganism control only:

1) The radiation source has to be Xenon lamps emitting wavelengths in the range of 200 to 1,100 nanometers (nm) with pulse duration no longer than 2 milliseconds (or ms).
2) The total cumulative treatment shall not exceed 12.0 Joules/square centimeter (or J/cm^2).
3) The treatment has to be minimal designed reasonably to accomplish the intended effect.

2.8 Application in food

Numerous studies have been done by different research group on the effect of UV light on different food items. In this review, some of the most relevant research activities in fresh vegetables and how the quality changes affected are discussed.

2.8.1 UV-C applications

Last two decades has seen the exposure of horticultural crops to artificial UV-C light as an efficient alternative to chemical fungicides for control for postharvest diseases in horticulture crops at 180-280 nm with maximum at $\lambda = 254$ nm (Rodov et al., 1992). UV-C has also been observed to induce post-harvest rot resistance in fruits and vegetables (Marquenie et al., 2002) and extending the shelf life by delaying the ripening. It is lethal to most microbes and do not leave residues in treated food (Bintsis et al., 2000). Though it offers many advantages, still it requires more research to optimize UV light.

Non-ionizing, germicidal UV-C light can be effectively used for decontamination of whole or fresh-cut fruits and vegetables as it severely affects the physiological processes in tissues and the microbial DNA gets damaged (Lucht et al., 1998). The process was reported by Lado and Yousef (2002). They stated that UV-C radiation generates hydroxyl ions from water and hydrogen atoms from DNA components, sugars and bases are removed, which inhibits microbial growth. Pyrimidine dimers are formed at 254 nm UV which alters the DNA helix and blocks the microbial cell replication and the damaged cells which can't be repaired, die (Lado and Yousef, 2002).

Costa et al., (2005) treated Broccoli head with UV-C to obtain doses of 4, 7, 10 and 14 kJ/m^2 by placing the heads vertically in the chamber to ensure a homogeneous radiation on the surfaces. Broccoli heads were stored in dark at 20 °C for 5 days' post treatments covered with PVC films to protect any water loss during storage. The team observed delay in yellowing in all the treated samples. The sample treated with 10 kJ/m^2 observed to have highest retention of chlorophyll. It was confirmed that the treatment is effective in suppressing chlorophyll *a* and *b* degradation. Also, lower activity of chlorophyll peroxidase and chlorophyllase observed with the treated samples. It was concluded that UV light treatment was causing lower respiration rate and higher antioxidant capacity.

Allende et al. (2006) studied the effectiveness of UV-C treatment on fresh minimally processed "Red Oak Leaf" lettuce by treating on both the sides. UV-C light in the region of 220-

290 nm was used to treat the lettuce at different energy level (1.18, 2.37 and 7.11 kJ/m^2) by adjusting the exposure time. Treatment was given at 10^0C and stored after treatment at 5 °C for 10 days. The samples were analyzed for 20 different microbial groups from different genera often associated with fresh produce (*Enterobacter, Erwinia, Escherichia, Leuconostoc, Pantoea, Pseudomonas, Rahnela, Salmonella, Serratia and Yersinia*). UV-C light energy as low as 30 J/m^2 was sufficient enough to inhibit the growth of most of the bacteria strains. For some resistant strain like *Erwinia carotovora, Leuconostoc carnosum, Salmonella typhimurium, and Yersinia aldovae* higher energy of 85 J/m^2 was enough for complete inhibition of growth. Based on the results, it was seen higher the radiation dose, greater the microbial inhibition. The overall shelf life was observed to extend by 2 days than the untreated samples.

Liu et al. (2012) observed 92.83% increase in antioxidant value when mature-green tomatoes are treated with UV-C of 4 kJ/m^2. In the same experiment, the effect on total phenolic contents were studied and found to increase by 21.2% and 20% with treatment of 4 and 8 kJ/m^2 respectively. Robles et al. (2007) found delay in ripening in fresh tomatoes and better sensory attributes when treated with 4 kJ/m^2 under controlled storage condition. Yaun et al. (2014) studied pathogenic bacteria *Salmonella* and observed 2.19 log reduction in *Salmonella* species when treated with 253.7 nm at 1.5 - 24 mW/cm^2 energy.

Erkan et al. (2001) demonstrated that when zucchini squash slices were exposed to UV light for 10 and 20 min, it reduced the deterioration of the slices as well as microbial activity during storage at 5-10 °C. Higher respiration rates were noted and the chilling injury which is dried sunken brown spots on the surface was observed only after 20 days of storage at 5 °C. Before 20 days, no consistent effect was observed on sugar or malic acid concentrations. The most pronounced effect of UV-C irradiation noted was retardation of the microbial growth, thus, providing a basis for the frequently observed delay in senescence and subsequent deterioration in fruit tissues.

Yaun et al. (2004) investigated the bactericidal effect of UV-C light at 253.7 nm with 1.5 to 24 mW/cm^2 dose range by inoculating red apples, leaf lettuce and tomatoes with cultures of Salmonella species or *Escherichia coli* O157:H7. Different log reductions, varying from 2.19 logs of Salmonella species for tomatoes to 3.3 logs for apples inoculated with *E. coli* O157:H7, were obtained at the highest dose of UV-C light of 24 mW/cm^2. The differences in value could be due to bacterial shielding from the UV light by the wax applied on the tomato surface. Though, no significant difference on the green lettuce surface was observed.

Similar study was performed for apple cider as there were concerns regarding food safety and contamination of unpasteurized apple cider from *E. coli* O157:H7. UV light pasteurization can be used as a low-cost alternative to conventional method of pasteurization using heat for small processing plants. A study was conducted on raw unpasteurized apple cider to observe efficacy of UV treatment with respect to the exposure time and dosage of treatment by observing physical parameters. UV light at 254.7 nm was used at dosage of 8777 μW-s/cm^2 to reduce bacteria inoculated apple cider at 2.20 logs per pass and exposure was timed at for 2.03 s (single pass), 4.06 s (double pass), 6.09 s (triple pass) and 8.12 s (four pass), respectively, at a flow rate of 6.30 L/min. In most of the cases, 5-log reduction was achieved, and it was in compliance with FDA. UV treated apple cider experiments observed a significant shelf life extension by inhibiting growth of yeast and mold. Also, it was observed as a viable cost-effective alternative for low throughput apple cider processing operations and the log reduction of yeast, mold and bacteria were associated with turbidity, color and viscosity of apple cider. The cider became dark with exposure to UV and turbidity was observed to decrease, though no significant differences in sensory properties was noted (Donahue et al., 2004).

Fonseca and Rushing (2006) reported the influence of UV-C light at 1.40-13.70 kJ/m^2 on the quality of fresh-cut watermelon. Watermelon was exposed to UV light at 4.1 kJ/m^2 and it produced more than 1 log reduction in microbial populations without affecting juice leakage, color, and overall visual quality (Fonseca and Rushing, 2006).

Schenk et al. (2008) explored the microbicidal effect of UV-C light (λ = 253.7 nm) at dose ranged between 0 and 87 kJ/m^2 on pear slices peeled and unpeeled against Listeria innocua ATCC 33090, Listeria monocytogenes ATCC 19114 D, Escherichia coli ATCC 11229, and *Zygosaccharomyces bailli* NRRL 7256 and these along with some others were used for inoculation. The pear slices were inoculated and treated with UV-C. The dose of UV light was increased by increasing the time of exposure and it resulted in efficient inactivation of these microbes. The effect was observed to be more prominent for peeled pear slices (Schenk et al., 2008).

The treatment with UV light during pre-storage was also observed to be effective in controlling fungal decay in variety of fruits and vegetables. For instance, a study was performed by Baka et al. (1999) regarding the effect of pre-storage exposure to shortwave ultraviolet (UV-C) light on the decay and quality of fresh strawberries. They exposed fresh strawberries to UV-C at 0.25 and 1.0 kJ/m^2 and UV-treated fruits were packed in plastic mesh baskets and were stored

in the dark at 4 °C or 13 °C with 95% relative humidity created by continuous ventilation with humidified air. UV-C treatment was used to control the decay caused by Botrytis cinerea at both temperatures of study for 4 to 5 days. Lower respiration rates, higher anthocyanin content and titratable acidity was observed with fruits treated with UV and were firmer in texture. Though the fruits treated with 0.25 kJ/m^2 had a slower rate of senescence, the maximum dose of 1.0 kJ/m^2 caused damage to the fruits. Overall, UV-C exposure at 0.25 kJ/m^2 slowed the ripening and senescence of strawberries stored at 4 °C (Baka et al., 1999).

The effect of UV-C radiation was observed on cantaloupe melon at various conditions. The storage properties were observed for cut fruit at 10 °C and was then compared with post cut UV treated fruit and untreated control. A hypersensitive defense response was induced by UV treated cut fruit and increase in accumulation of ascorbate peroxidase was observed. Lowest levels of esterase and lipase activity was noted for fruit processed under UV-C radiation compared to post-cut treated fruit throughout the storage period. But after 7 days of storage, the lipase activity was completely undetectable and reduced respiration rates were observed. The study concluded that treatment with UV-C was effective in reducing yeast and molds and improved the shelf life of cut cantaloupe melon, further improving the product quality (Lamikanra et al., 2005). Lamikanra and Watson (2004) also showed that the post-cut treatment of cantaloupe melon with UV radiation lowers the esterase (ES) activity in fresh-cut fruit and maintained lower activity during storage (Lamikanra and Watson, 2004). This esterase activity reduction in processing of fruit under UV light could have been responsible for retention of fruity characteristics in cut fruits during storage. The lipase activity in fruit processed under UV-C light was lower than post-cut treated fruit but was comparable to the untreated fruit immediately after cutting.

Abiotic stresses such as heat shock and UV-C irradiation can be used to prevent decay in fresh-cut fruits and vegetables by inducing synthesis of bioactive compounds. A study was performed on whole carrots to evaluate the effects of heat shock and UV-C radiation stress treatments during storage at 5 °C. UV-C at 0.78 and 0.36 kJ/m^2 and heat shock at 100 °C for 45 s was applied to carrots and was compared to control samples during storage. Reduced carotenoid content was observed in all the samples, but UV samples registered a three-fold higher carotenoid content compared to control. Also, the respiratory metabolism was found to be affected by both abiotic stress treatments. A 2.5 Log10 CFU/g reduction in initial microbial load and reduced microbial growth were achieved by UV-C treatment. Also, the heat shock pre-

treatment was noted to be an effective alternative instead of using chlorine in industrial processing of fresh-cut carrots. This study concluded that combining both abiotic treatments than singular pre-treatments could be more effective (Alegria et al., 2012).

Fresh produce is still a comparatively leading in foodborne illness outbreaks. Thus, there is a pressing need for an effective post-harvest decontamination intervention such as UV that can replace or supplement post-harvest washing in case of fresh produce. However, the commercial application of this technology has not been widely developed or applied in current days due to several limitations to this technology.

From the technological standpoint the concept of pulsed UV light is possible. In the pulsed light technology, the broad spectrum of the pulsed light can be replaced with only UV light. UV light is the source of energy in pulsed light system and UV-C is known for the germicidal effect. The combination of high intensity pulsed UV will be more effective in its germicidal effect. Currently, the concept of pulsed UV is not available commercially. However, it can be achieved by filtering the broad wavelength of the pulsed light with suitable filters.

2.8.2 PL applications

Murugasen et al. (2012) examined by applying pulsed light on mature and completely ripened elderberry fruit at different doses of energy to comprehend the effect of post PL treatment on phenolic compounds. As a major aspect of this test, elderberry was picked from the farm, cleaned and stored at refrigerated temperatures. Preceding the treatment, the samples were kept at room temperature to permit the berries to attain the room temperature. As a component of the treatment, four pulsed lengths (5, 10, 20 and 30 seconds) were applied at three energy levels (4500, 6000, 11,000 J/m^2/pulse). Elderberries were put away for 24 h at refrigerated temperature post treatment to permit proper development pf polyphenols and assessed. The study showed that a successful increase in the total phenolics by 50% and 40% in case of exposure with 11,000 J/m^2/pulse for 10 s and 11,000 J/m^2/pulse for a 5 s respectively. They additionally noticed interesting observation that increase in energy level from 4500 J/m^2/pulse to 6000 J/m^2/pulse resulted in decreasing the total phenolics in elderberries. The result demonstrated that the production of total phenolics due to the pulsed light stress is related to the intensity of the treatment.

Oms-Oliu et al. (2010) investigated the impact of PL on overall quality of fresh-cut mushrooms. Pulsed light energy of 4.8, 12 and 28 J/cm^2 were applied on mushrooms and

evaluated for microbial quality, browning, texture and antioxidant properties. The total microflora reduced from 0.6 to 2.2 log after 15 days of storage in refrigerated condition. The overall shelf life increased by 2-3 days' post treatment as compared to untreated mushrooms. Treatment at 12 and 28 J/cm^2 was observed to impact the texture of the mushrooms during storage due to thermal damage to the mushroom cells. Total phenolic compounds was also observed to be reduced at 28 J/cm^2 treatment dose. It was concluded that 4.8 J/cm^2 treatment is the most ideal treatment for quality enhancement without impacting the texture and antioxidant properties.

2.8.3 Comparative study of UV-C and PL

Comparative studies are also done by Pataro et al. (2015) on immature green tomatoes by exposing UV light energy using both pulsed light and UV-C irradiation of 1-8 J/cm^2 and further storing at 20 ± 2 °C for up to 21 days. The antioxidant properties were evaluated post treatment and with storage as compared to the untreated tomatoes. There was no influence of light treatment on the pH, °Brix and also on the overall change in color of the tomatoes. All the treated samples showed increase in lycopene, total carotenoids, phenolic compounds and antioxidant activity by 6.2, 2.5, 1.3, and 1.5-fold respectively. In this study UV-C light appeared to be more efficient than pulsed light in increasing the phenolic compounds.

Also, compared to continuous UV light, the pulsed light has been shown to be more effective for destruction and inactivation of microbes (Fine and Gervais, 2004). Another study was performed to test pulsed light treatment on three varieties of tomatoes (Micron, BNC8015 and AA7033) and two varieties of peaches (Lucia and Duceur). The experiment was conducted at different energy doses (1, 2, 4, and 8 J/cm^2/side). The lethality of pulsed light treatment on yeast and mold population was assessed by the total aerobic mesophilic count and reduction of native microflora was observed which increased progressively with increasing the energy incident on the surface of each product. Though, no complete inactivation occurred even when the higher energy dose was applied. The sensitivity of the treatment varied with variety of the product. Micron variety highlighted greater sensitivity to light pulses than variety BNC8015 and AA7033. Between the two varieties of peaches, Lucia variety showed the higher sensitivity to light pulses. Thermal damage was observed on peaches when treatments of higher energy dose were applied. Therefore, multi-step treatment in which each side of the product was exposed

several times to light pulses of low energy dose (31.4 J/cm^2) made it possible to achieve the same lethal effect along with the minimum thermal damage /side) per step (Pataro et al., 2015).

Pulsed light treatment is used as a new method to maintain physical and nutritional quality of fresh-cut fruits. A study was conducted on fresh cut mangoes to prove the same. The study looked at the impact of pulsed light treatment on quality of fresh cut “Kent” mangoes. Pulsed light treatments were carried out using an automatic flash Xenon lamp system (Mulieribus, Claranor, France) which was composed of eight lamps situated all around the sample with a total fluence of 8 J cm^2 for uniform exposure. Various parameters like color, phenol, carotenoid, ascorbic acid contents and enzyme activities were analyzed. The firmness, color and carotenoid content were preserved with the pulsed light treatment while the conventional methods like thermal treatment causes degradation of some components (Charles et al., 2013)

It is evident from the above literature reviews that both UV-C light and pulsed light technology can induce quality improvement in fresh produce when treated at the appropriate energy level. There are also certain adverse quality changes when exposed to an intense or higher energy level, so this aspect requires research on finding the most favorable energy level. Hence this research was focused on the evaluation of the effects of PL and UV-C treatments on vegetable shelf life and quality enhancement.

PREFACE TO CHAPTER 3

In many parts of the world there is a recent shift toward healthy living and consumption of healthier foods among consumers. This has increased the popularity of including fruits and vegetables in the diet which can be mostly in raw form, in salads and sandwiches or in different forms. Fruits and vegetables are vital to our health and well-being, providing essential vitamins, minerals and fiber. Recently, however, the fresh-cut fruits and vegetables available in the market continues to be the main source of foodborne illness outbreaks implicating pathogens such as *Escherichia coli* O157:H7, *Salmonella*, *Listeria monocytogenes* and human parasites (e.g. *hepatitis* A, *Cyclospora*). These foodborne illness outbreaks result in sickness, hospitalizations, and deaths of consumers, as well also have a serious adverse economic impact on growers and processors. The current challenges ensuring the safety of fresh food produces are to prevent contamination in the field and to minimize cross-contamination during post-harvest handling. Preventing contamination in fields or greenhouses is challenging and even good agricultural practices (GAP) are insufficient to ensure human pathogens are not introduced into the fresh produce chain. A more effective means of control is to apply post-harvest decontamination interventions to enhance the microbiological safety of fresh produce. The focus of this chapter is to evaluate the effectiveness of pulsed light (PL) technology (novel non-thermal method capable of microbial surface decontamination of foods) treatment to fresh carrots (whole and cut form) at different selected pulsed light doses within the FDA recommended limit of not more than 12 J/cm^2 on food surfaces. PL treated carrots (both whole and cut) were monitored for qualitative changes upon storage for 28 days under different storage conditions.

All experimental work and data analysis were conducted by the candidate under supervision of Dr. H. S. Ramaswamy. A part of this research was presented in the form of poster at CTAQ meeting June 2018:

Prusty, P., Ramaswamy, H.S., 2018. Effectiveness of pulsed light (PL) and UV-C light treatments on the quality and shelf-life of fresh carrot and radish. Poster presented at CTAQ meeting June 2018.

CHAPTER 3
STUDY OF QUALITY AND SHELFIFE ENHANCEMENT OF FRESH CARROTS WITH PULSED LIGHT TREATMENT

Abstract

The purpose of designing this experiment is to evaluate the pulsed light (PL) treatment effects on overall quality and shelf life of fresh whole and cut carrots at different treatment levels (within the FDA approved permissible limit for use on foods) and storage conditions. Fresh whole carrots were purchased on the same day from the local grocery store. The carrots were sorted out to remove the damaged, followed by the washing and then air-dried prior to the PL treatment. Different levels of PL treatment were given to both whole and cut carrots. The data obtained were used to characterize the significance of PL treatment in terms of defined shelf life and quality parameters such as physiological changes, pH changes, moisture loss, antioxidant activity, peroxidase activity and microbiological study (aerobic, yeast and mold) of log reduction. The study revealed that PL treatment did not significantly increase the overall shelf life of carrots. However, the study demonstrated a significant positive impact on quality parameters. PL treatment caused lower changes in pH for whole carrots. Moreover, for both (cut and whole carrots), peroxidase enzyme did not get deactivated post treatment, while the antioxidant activity of whole carrots showed an increase of 85% after treatment with PL at 11.25 J/cm^2 at the storage environment. Whereas, for the cut carrots after PL treatment of 1 s and 3 s showed 89% and 91% increase in antioxidant activity. Also, the higher log reduction was achieved for aerobic and yeast & mold count. Hence, a PL treatment found its usefulness as a novel, non-thermal surface microflora decontamination technology for food products to achieve effective inactivation of micro-organisms.

3.1 Introduction

Fresh produce short shelf life is the biggest challenge in produce industry. Consequently, it leads to waste problem in food supply chains. Food can be wasted due to quality standards, which reject food items not perfect in shape or appearance. At the consumer level, insufficient purchase planning and expiring ‘best-before-dates’ also cause large amounts of waste, in combination with the careless attitude of those consumers who can afford to waste food. The

causes of food losses and waste in low-income countries are mainly connected to financial, managerial and technical limitations in harvesting techniques, storage and cooling facilities in difficult climatic conditions, infrastructure, packaging and marketing systems. Food waste in industrialized countries can be reduced by raising awareness among food industries, retailers and consumers. There is a need to find good and beneficial use for safe food that is presently thrown away (FAO, 2011).

As a key to the food waste problem, there is a trend towards developing shelf life extension solutions that are intended to allow products not only to last longer but also to improve their quality and nutritional benefits (Gadde and Amani, 2016). The objective of this study was to evaluate the effect of PL treatment on overall quality and shelf life of fresh whole and cut carrots.

3.2 Materials and Methods

3.2.1 Materials

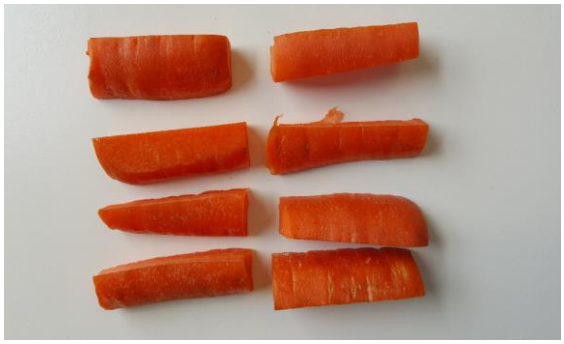
Fresh whole carrots (Figure 3.1a) from local grocery store were obtained on the day of PL treatments. The carrots were washed and cool air dried prior to use. Damaged or blemished carrots were sorted out. Whole carrots were cut in to 5 cm length and 3-4 cm width for cut carrot treatments (Figure 3.1b).

3.2.2 Research methods

3.2.2.1 Treatment of pulsed light

PL treatment is given using a R&D Benchtop pulsed light system - Steripulse-XL (RS-3000C, Xenon Corp., Wilmington, Mass., U.S.A.) (Figure 3.2). The sample was placed on the center of a glass container (bottom covered with aluminum foil for maximum light reflection onto the sample) and subjected to following treatment times: 1 pulse, 2 pulses and 4 pulses on each side of the sample.

(a) Fresh whole carrots (Length = 10-14 cm, Weight = 45-60 g)



(b) Fresh cut carrots (Length = 5 cm, Width = 3-4 cm, Weight = 6-8 g)

Figure 3.1 Fresh whole & cut carrots

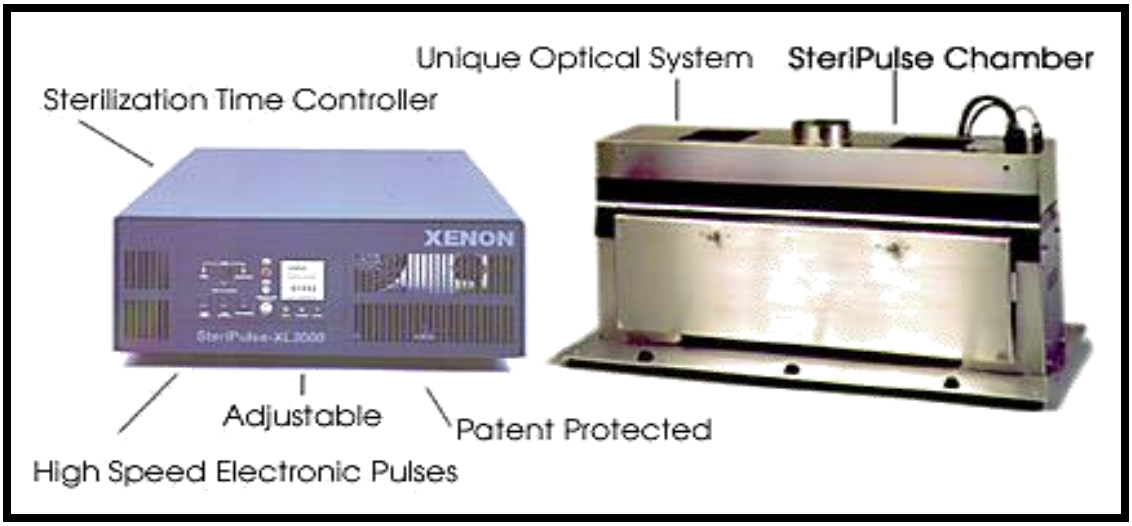


Figure 3.2 SteriPulse-XL 3000 Pulsed Light Sterilization System

The Steripulse–XL is a PL treatment equipment comprising of a high energy system used for decontamination of food, medical devices, packaging materials, blood or pharmaceutical products. The equipment is equipped with pulsed UV/Visible lamps capable of delivering 505 Joules/pulse which converts into a maximum of 1.27 J/cm^2/pulse on the middle tray located at 4th position from the top among 7 available locations. Excessive heat buildup is prevented by using very short pulse time width (360 μs). The equipment is pre-programmed with 3 pulses per second on a treatment time basis and could be stopped at any given number of pulses. Since the PL source lamp is made of Xenon, there is no hazardous risk as compared to the mercury lamps used in traditional UV lamps. The treatment chamber is equipped with forced air evacuation mechanism to ensure no heat buildup from the lamp. The forced air has filters capable of filtering microbes and ozone resistant at both the ends. The chamber has interlocking mechanism with a safety interlock switch to prevent undesirable exposure to the operator. The chamber is made of stainless steel for easy sterilization and cleaning.

The sterilization system is connected with a controller to control the pulse application. The pulse rate is 3 pulses/s (set by the manufacturer) and pulse mode can be either timed or continuous. The controller has a programmable timer with a range from 1 to 999 s at 1 second interval. The sterilization chamber has racks at different heights and the energy exposure level varies depending on the location inside the chamber (Figure 3.2).

3.2.3 Experimental design

Fresh whole carrots were treated with PL fluence (within the FDA approved permissible limit for use of PL to foods) of 11.25 J/cm^2 while fresh cut carrots were subjected to two fluence levels of 2.25 and 6.75 J/cm^2. Different levels of energy application had been designed and applied based on the FDA guidelines (CFR - Code of Federal Regulations Title 21) for the maximum exposure level by using the PL system. The experiment has designed with 2 different factors, which are treatment and storage. Each factor has further divided into 3 levels. Samples were stored at three different storage conditions (room temperature, refrigerated 10-15 °C, and 0-4 °C). Fresh whole and cut carrots were treated according to the experimental design and compared with a control set without treatment.

Evaluation was carried out on Day 0 for appearance, total solids, pH, antioxidant activity, total aerobic count, and yeast & mold count to compare the qualitative changes with treatments

as compared to without treatment. Similar quality parameters were evaluated by repeating at day 7, day 14, day 21, and day 28 to monitor the changes in quality with time at different storage conditions. PL treatment and storage condition (Table 3.1) were followed as below for fresh carrots:

Table 3.1 Experimental design set point for treatment of carrots

Carrot	Pulsed light treatment	Storage
Fresh Whole	11.25 J/cm^2	Room Temperature, Refrigerated (0-4 °C and at 10-15 °C)
Fresh Cut	2.25 and 6.75 J/cm^2	Room Temperature, Refrigerated (0-4 °C and at 10-15 °C)
Control	No treatment	Room Temperature, Refrigerated (0-4 °C and at 10-15 °C)

3.2.4 Instruments

The following instruments were used for the treated sample analysis:

3.2.4.1 UV-Vis spectrophotometer

UV-3100PC (VWR International, LLC., USA), UV/VIS scanning spectrophotometer with a spectral bandwidth of 2 nm was used. The light source of the spectrophotometer is a deuterium/tungsten halogen lamp with a stray light of ±0.05% T at 220, 360 nm and wavelength range of 190-1100 nm. The spectrophotometer is equipped with an application software which allows PC control of the spectrophotometers and includes the following methods: basic mode, quantitative, wavelength scan, kinetics, multi-wavelength and DNA/protein. Among these methods, wavelength scan method was used for the measurement studies.

3.2.4.2 pH meter

Accumet AB 15\ 15+ bench-top meter (Fisher Scientific Company, USA) was used for analysis of pH of the samples. This meter consists of an electrode arm with metal bracket, 110/220 universal power supply and USB cable. It is capable of providing microprocessor precision in a compact benchtop design which is easy to use. The meter allows measuring the

pH, absolute mV or relative mV or temperature as desired. It allows to select one of three sets of standard buffer groups as well as to standardize with up to five/six buffers.

3.2.5 Analysis

The treated samples were then subjected to following analysis:

3.2.5.1 Moisture loss

As per the oven-drying method for moisture determination, an accurately weighed quantity of the test sample was dried in a hot air oven for 24-48 h at 110 °C till no change in weight was observed. Total solids content was calculated by knowing the initial and final weight of the sample as a percentage (final weight / initial weight x 100). The difference between 100 and the total percentage solids was taken as the percentage moisture content.

3.2.5.2 pH

As per the potentiometric titration, 10 g of treated samples were taken and homogenized with 20 ml water. pH of the homogenized sample mixture was determined using Accumet AB 15\ 15+ bench-top meter by Fisher Scientific.

3.2.5.3 Antioxidant activity

Total antioxidant capacities of carrots were quantified using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay (Singh and Ramaswamy, 2014) with some modifications. Methanolic extracts of samples were prepared by adding 50 ml of HPLC grade methanol to 2 g pureed sample. The samples were then vortexed and centrifuged for 25 min at 4000 rpm. The centrifuged samples were then filtered using whatman No 1 filter paper. An aliquot of 8 ml of Methanolic extract was added to 6 mL of DPPH in ethanol (0.01 g/100 ml), and the decolorization of DPPH solution was allowed to occur when kept for 30 min in the dark. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The absorbance of sample was then measured by UV-Vis spectrophotometer at 517 nm. The scavenging activity percentage (% AA) was determined using the following formula [with As, Ab and Ac representing absorbance values for sample, blank and control, respectively]:

$$\% AA = 100 - \{[(As-Ab) \times 100] / Ac\} \qquad \text{- - - - - (Eq 3.1)}$$

3.2.5.4 Peroxidase activity

Peroxidase activity of carrots were conducted using a simple enzyme activity check method (Dauthy, 1995). 25 ml solution A of 1% guaiacol solution (Fisherscientific company, Canada) in 95% ethyl alcohol (Fisherscientific company, Canada) was prepared followed by 25 ml solution B of 0.3% hydrogen peroxide (Fisherscientific company, Canada) in water and solution C was prepared by mixing 1 ml solution A with 1.6 ml solution B in test tube. Later, 10 g of sample was cut and added into 20 ml water followed by blending until resulted into smooth. Further, solution C was added in the blended sample and mixed thoroughly. A rapid and intensive brown-reddish color development of the mixture indicates a high peroxidase activity (positive reaction).

3.2.5.5 Microbiological analysis

Microbiological analysis of carrots was performed by determining the total aerobic mesophilic microorganisms and yeast and mold populations based on the methods mentioned by Gómez et al. (2012). 2 g of treated samples were homogenized using sterile distilled water. Appropriate serial dilutions were performed in 0.1% sterile peptone water (Difco) and spiral plated on Potato Dextrose Agar and Difco™ Plate Count Agar (Fisherscientific company, Canada) to determine the yeast & mold and total aerobic plate count of the samples respectively. The plates were then incubated for 48 h at 37 °C for total aerobic microorganisms and at 25 °C for 5 days for yeast and mold after which the colonies were enumerated.

3.2.5.6 Statistical data analysis

The statistical data analysis was carried out for interpretation of the designed experiment (section 3.2.3). The “null hypothesis” is the proposition that there is no effect or no relationship between phenomena or populations, whereas the “alternative hypothesis” states that a population parameter is smaller, greater, or different than the hypothesized value in the null hypothesis. The alternative hypothesis is tested to be true or not.

The following steps were followed to study the effect of treatment on the parameters:

Step 1: First, an **F-Test** was performed to determine if the variances of the two populations are equal.

If F > F Critical one-tail, the null hypothesis was rejected and the alternative hypothesis was accoeted. This meant, the variances of the two populations were unequal.

If F < F Critical one-tail, the null hypothesis was accepted. This meant, the variances of the two populations can be assumed to be equal or unequal.

Step 2: Select a **t-Test**: Two sample assuming equal or unequal variance from the result of step 1.

s = Significant

[If **t stat** does not comply, **-t Critical two-tail < t Stat < t Critical two-tail**, then reject the null hypothesis]

ns = Not significant
[If **t stat** does comply, **-t Critical two-tail < t Stat < t Critical two-tail**, then do not reject the null hypothesis]

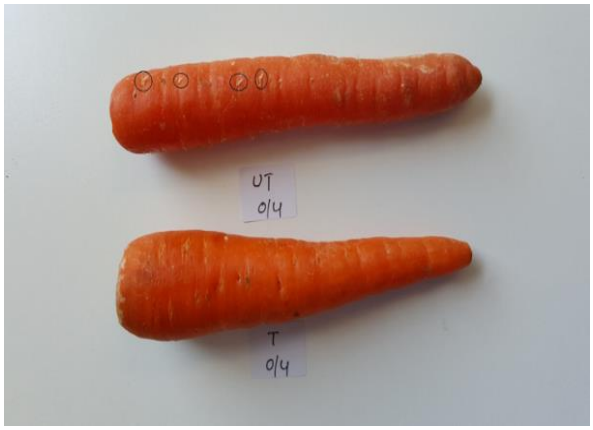
3.3 Results and Discussion

3.3.1 Effect of PL Treatment on shelf-life of Whole and Cut Carrot

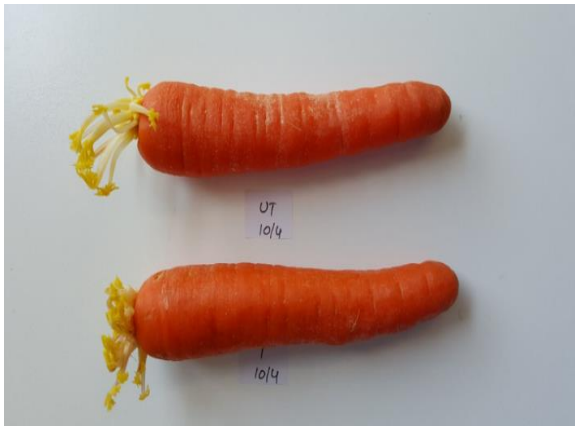
The visualization study of whole carrots and cut carrots were carried out to study the overall effect of the treatment on shelf-life. Different quality parameters were evaluated within an acceptable condition of the carrots and explained as below.

3.3.1.1 Physiological changes in whole carrots with storage time

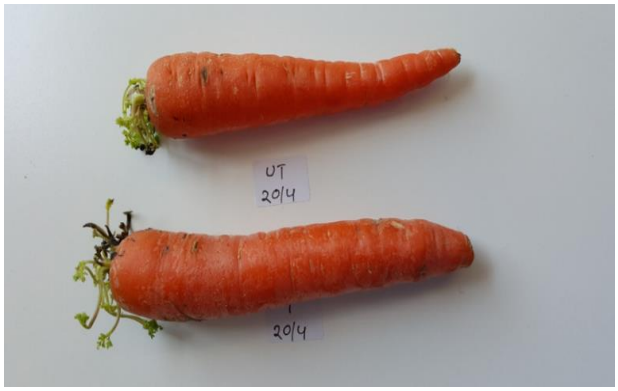
The Figures 3.3a, 3.3b and 3.3c demonstrate the visual appearance of treated and untreated whole carrots stored at each storage conditions at the end of 4^{th} week. there was no visible difference between treated and untreated whole carrot when stored at 0-4 °C at the end of 4^{th} week (Tables 3.2 & 3.3).



A: Refrigerated (0-4 °C)



B: Refrigerated (10-15 °C)



C: Room temperature

Figure 3.3 Physiological study observation for whole carrots

However, at the end of 4th week, both treated and untreated whole carrot showed yellow germination when stored at 10-15 °C and the green germination when stored at room temperature. No other physiological difference was observed at the end of week 4.

3.3.1.2 Physiological changes in cut carrots with storage time

The study revealed that in case of treated cut carrots, rapid spoilage was noticed. The study was concluded at the end of 2nd week for all the samples that were stored at room temperature and refrigerated temperature (0-4 °C and 10-15 °C).

Table 3.2 Visual study results for whole carrots

Sample identification (Whole carrots)	Observations				
	Week 0	Week 1	Week 2	Week 3	Week 4
Untreated (Room temperature)	ND	ND	ND	ND	D
Treated (Room temperature)	ND	ND	ND	ND	D
Untreated (Refrigerated 0-4 °C)	ND	ND	ND	ND	ND
Treated (Refrigerated 0-4 °C)	ND	ND	ND	ND	ND
Untreated (Refrigerated 10-15 °C)	ND	ND	ND	ND	D
Treated (Refrigerated 10-15 °C)	ND	ND	ND	ND	D

Treated = Pulse light treatment at 11.25 J/cm^2; ND = No visual difference
D = Visual difference (Spoilage indication, germination, color change, spot observation)

Table 3.3 Visual study results for cut carrots

Sample identification (Cut carrots)	Observations			
	Week 0	Week 1	Week 2	Week 3
Untreated (Room temperature)	ND	ND	ND	-
Treated 1 (Room temperature)	ND	ND	D	-
Treated 2 (Room temperature)	ND	ND	D	-
Untreated (Refrigerated 0-4 °C)	ND	ND	ND	-
Treated 1 (Refrigerated 0-4 °C)	ND	ND	D	-
Treated 2 (Refrigerated 0-4 °C)	ND	ND	D	-
Untreated (Refrigerated 10-15 °C)	ND	ND	ND	-
Treated 1 (Refrigerated 10-15 °C)	ND	ND	D	-
Treated 2 (Refrigerated 10-15 °C)	ND	ND	D	-

Treated 1 = Pulse light treatment (time of 1 s) at 2.25 J/cm^2
Treated 2 = Pulse light treatment (time of 3 s) at 6.75 J/cm^2
ND = No visual difference
D = Visual difference (Spoilage indication, germination, color change, spot observation)
- = No observation (Samples were thrown out due to complete spoilage)

The week 2 results at room temperature showed visible spoilage on cut treated carrots, while untreated carrots did not show any symptoms of spoilage. This would have been due to the severity of the treatment which caused damage to the exposed cells of the carrots (Gonzales and Barrett, 2010) (Figure 3.4).

A: Room temperature B: Refrigerated (10-15 °C)

C: Refrigerated (0-4 °C)

Figure 3.4 Physiological study observation for cut carrot

3.3.2 pH changes with storage time

Measurement of pH can help understand the chemical changes (in terms of spoilage indicator) during storage. Therefore, whole and cut treated carrots were monitored to study the effect of change in pH throughout the storage compared with untreated control sample. The mean pH values of the whole treated and whole untreated carrots are presented in Table 3.4, which is ranged between 5.74 and 6.29.

The followed trend showed faster drop in pH value of untreated whole carrots compared to treated whole carrots stored at room temperature, 10-15 °C and 0-4 °C for the shelf-life study period of 4 weeks. Figure 3.5 depicts the average pH values of the whole treated and whole untreated carrots.

Table 3.4 Changes in pH of treated with untreated whole carrots

Storage Temperature	**Whole Carrot Type**	**Week 0**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Room Temperature	Untreated[a1]	6.28 (± 0.03)	6.14 (± 0.02)	6.05 (± 0.03)	5.93 (± 0.04)	5.74 (± 0.05)
	Treated[a2]	6.26 (± 0.03)	6.17 (± 0.03)	6.15 (± 0.02)	6.05 (± 0.04)	5.94 (± 0.04)
10-15 °C	Untreated[b1]	6.24 (± 0.04)	6.13 (± 0.03)	6.04 (± 0.02)	6.01 (± 0.03)	5.91 (± 0.04)
	Treated[b2]	6.28 (± 0.03)	6.18 (± 0.02)	6.16 (± 0.05)	6.08 (± 0.02)	6.00 (± 0.05)
0-4 °C	Untreated[c1]	6.26 (± 0.03)	6.20 (± 0.02)	6.10 (± 0.03)	6.04 (± 0.03)	5.98 (± 0.03)
	Treated[c2]	6.29 (± 0.02)	6.22 (± 0.03)	6.16 (± 0.03)	6.12 (± 0.02)	6.03 (± 0.02)

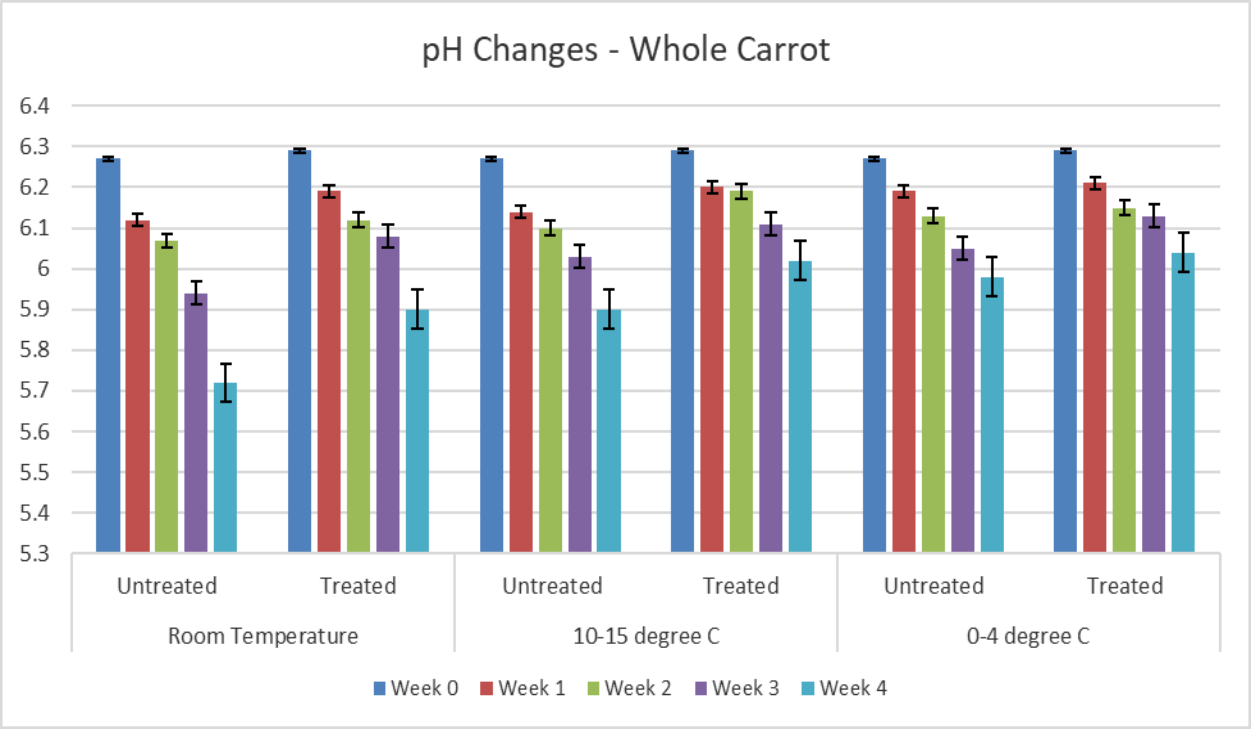


Figure 3.5 Changes in pH comparison of treated whole carrots sample with untreated sample

The primary reason for the rapid pH drop is the faster spoilage of untreated carrots. In the case of PL treated whole carrots, the slower pace in drop of pH values was observed. This could be due to PL treatment caused the inactivation of the spoilage organism. Thus, the overall study result showed a lower rate of pH drop for whole carrots was achieved by PL treatment. The statistical analysis results for whole carrots are mentioned in Table 3.5.

Table 3.5 t-Test: Two-sample assuming equal or unequal variances for whole carrots

Storage	Whole Carrot types	Variance (F test)	t-stat	t Critical two-tail
Room temperature	a1-a2 (ns)	Equal	-0.80	2.31
10-15 °C	b1-b2 (ns)	Equal	-0.97	2.31
0-4 °C	c1-c2 (ns)	Equal	-0.72	2.31

The study results for treated and untreated cut carrots are summarized in the Table 3.6 and statistical data in Table 3.7.

Table 3.6 Changes in pH of treated with untreated cut carrots

Storage Temperature	Cut Carrot Type	Week 0	Week 1	Week 2
Room Temperature	Untreated [a1]	6.28 (± 0.02)	6.12 (± 0.03)	6.04 (± 0.03)
	Treated 1 [a2]	6.29 (± 0.02)	6.03 (± 0.02)	5.83 (± 0.04)
	Treated 2 [a3]	6.28 (± 0.02)	6.02 (± 0.03)	5.77 (± 0.03)
10-15 °C	Untreated [b1]	6.28 (± 0.02)	6.11 (± 0.02)	6.06 (± 0.04)
	Treated 1 [b2]	6.28 (± 0.02)	6.07 (± 0.03)	6.03 (± 0.03)
	Treated 2 [b3]	6.29 (± 0.02)	6.08 (± 0.02)	6.01 (± 0.03)
0-4 °C	Untreated [c1]	6.26 (± 0.02)	6.17 (± 0.02)	6.13 (± 0.02)
	Treated 1 [c2]	6.28 (± 0.02)	6.08 (± 0.03)	6.02 (± 0.02)
	Treated 2 [c3]	6.28 (± 0.02)	6.04 (± 0.02)	6.01 (± 0.03)

The graphical representation of the pH results for treated and untreated cut carrots shown in the Figure 3.6.

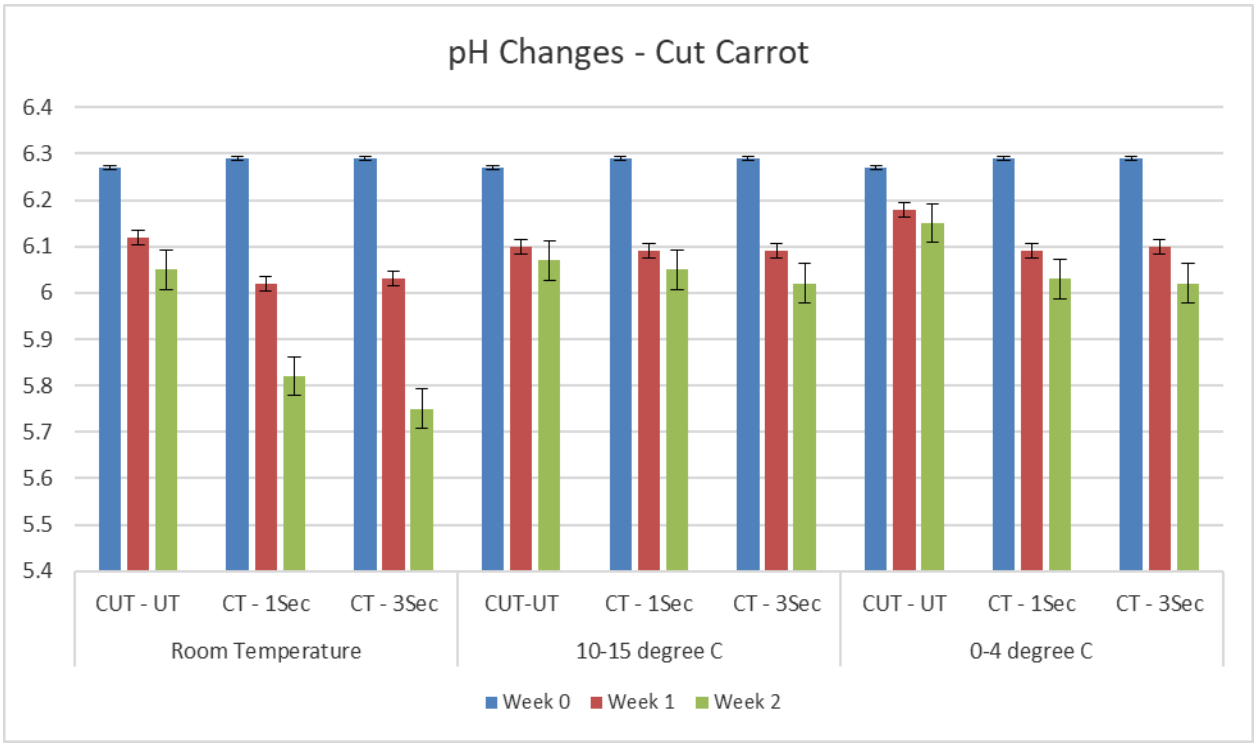


Figure 3.6 Changes in pH comparison of treated cut carrots sample with untreated sample

In the case of treated cut carrots stored at room temperature, significant drop in pH drop (from 6.28 to 5.77 for treated at 6.75 J/cm^2 and 5.83 for treated at 2.25 J/cm^2) than the untreated cut carrots (from 6.28 to 6.04) at the end of 2nd week. This is explained due to the lack of cell wall to protect the adverse effect. The intensity of the treatment contributed along with physiological damage to the exposed cells of cut carrots could have fueled the faster pH drop than untreated one. Therefore, the PL treatment in case of cut carrots leads to rapid quality deterioration in terms of rapid pH drop if stored at room temperature.

Table 3.7 t-Test: Two-sample assuming equal or unequal variances for cut carrots

Storage	Cut Carrot types	Variance (F test)	t-stat	t Critical two-tail
Room temperature	a1-a2 **(ns)**	Equal	-0.63	2.78
	a1-a3 **(ns)**	Equal	-0.74	2.78
10-15 °C	b1-b2 **(ns)**	Equal	-0.20	2.78
	b1-b3 **(ns)**	Equal	-0.22	2.78
0-4 °C	c1-c2 **(ns)**	Equal	-0.70	2.78
	c1-c3 **(ns)**	Equal	-0.80	2.78

3.3.3 Moisture loss with storage time

The study of moisture loss helps to understand the effect of treatment on the physical property of the carrots. Table 3.8 represents the percentage moisture values of the whole treated and whole untreated carrots.

In the case of whole carrots, the study results of the change in moisture percentage at three different storage conditions did not show any trend of PL treatment. However, faster rate of moisture change was observed towards the end of storage period at week 4 for the whole carrots stored at 10-15 °C temperature. The primary explanation for rapid moisture loss due to physiological and microbiological spoilage of carrots. The statistics are shown in Table 3.9.

Table 3.8 Percentage moisture change in treated with untreated whole carrots

Storage Temperature	Whole Carrot Type	Week 0	Week 1	Week 2	Week 3	Week 4
Room Temperature	Untreated[a1]	62.95 (±1.63)	62.63 (±0.61)	63.82 (±1.19)	61.10 (±0.20)	57.2 (±1.47)
	Treated[a2]	64.57 (±0.81)	60.97 (±0.33)	64.27 (0.66)	63.34 (±0.79)	59.62 (±0.89)
10-15 °C	Untreated[b1]	62.95 (±1.37)	61.19 (±0.14)	59.88 (±0.33)	59.84 (±0.54)	58.36 (±0.48)
	Treated[b2]	64.57 (±1.44)	62.21 (±0.33)	64.99 (±1.58)	63.46 (±2.20)	62.52 (±0.72)
0-4 °C	Untreated[c1]	62.95 (±1.41)	60.50 (±0.41)	67.90 (±1.44)	62.81 (±1.17)	61.25 (±0.10)
	Treated[c2]	64.57 (±0.66)	61.91 (±1.23)	65.16 (±0.69)	61.79 (±0.08)	61.02 (±0.30)

Table 3.9 t-Test: Two-sample assuming equal or unequal variances for whole carrots

Storage	Whole Carrot types	Variance (F test)	t-stat	t Critical two-tail
Room temperature	a1-a2 (ns)	Equal	-0.67	2.31
10-15 °C	b1-b2 (s)	Equal	-3.29	2.31
0-4 °C	c1-c2 (ns)	Equal	0.13	2.31

Similarly, no trend was observed for the percentage change in the moisture for cut carrots (Table 3.10). But rapid moisture loss noticed towards the end of week 2 for room temperature storage.

Table 3.10 Changes in percentage moisture of treated with untreated cut carrots

Storage Temperature	Cut Carrot Type	Week 0	Week 1	Week 2
Room Temperature	Untreated ^{a1}	37.05 (± 0.18)	32.78 (± 0.52)	30.89 (± 0.44)
	Treated 1 ^{a2}	35.43 (± 0.60)	35.66 (± 0.88)	32.79 (± 0.37)
	Treated 2 ^{a3}	35.43 (± 0.52)	33.26 (± 1.04)	31.54 (± 0.69)
10-15 °C	Untreated ^{b1}	37.05 (± 0.58)	36.05 (± 0.68)	35.34 (± 0.79)
	Treated 1 ^{b2}	35.43 (± 1.34)	38.41 (± 1.01)	41.20 (± 1.72)
	Treated 2 ^{b3}	35.43 (± 1.05)	35.44 (± 0.95)	39.81 (± 0.25)
0-4 °C	Untreated ^{c1}	37.05 (± 0.64)	34.01 (± 0.02)	35.26 (± 0.02)
	Treated 1 ^{c2}	35.43 (± 1.16)	36.97 (± 0.71)	36.13 (± 0.92)
	Treated 2 ^{c3}	35.43 (± 1.41)	36.80 (± 0.57)	34.75 (± 0.42)

The graphical representation of the study results for percentage moisture change in treated and untreated cut carrots shown in the Figure 3.7. The statistics are shown in Table 3.11.

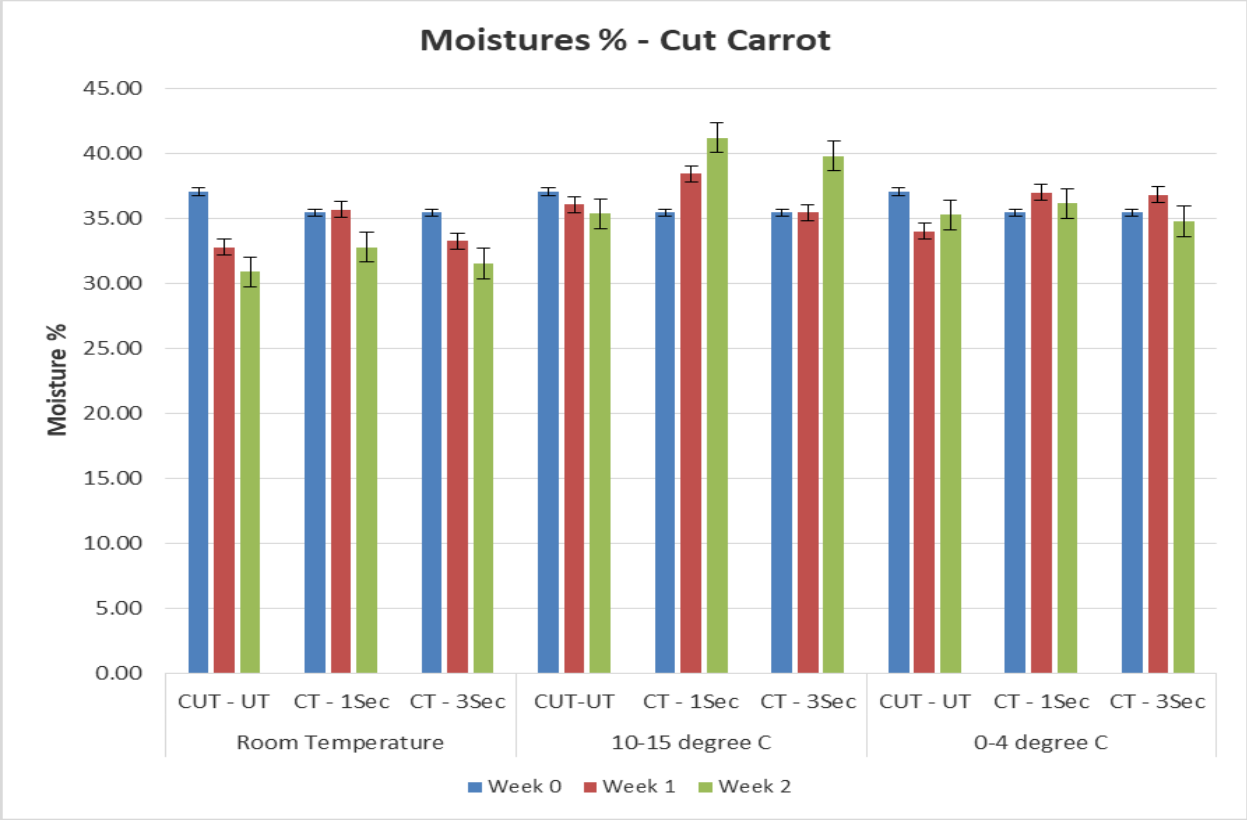


Figure 3.7 Changes in % moisture comparison of treated cut carrot sample with untreated sample

Table 3.11 t-Test: Two-sample assuming equal or unequal variances for cut carrots

Storage	Cut Carrot types	Variance (F test)	t-stat	t Critical two-tail
Room temperature	a1-a2 (ns)	Equal	-0.52	2.78
	a1-a3 (ns)	Equal	0.08	2.78
10-15 °C	b1-b2 (ns)	Equal	1.27	2.78
	b1-b3 (ns)	Equal	0.49	2.78
0-4 °C	c1-c2 (ns)	Equal	-0.75	2.78
	c1-c3 (ns)	Equal	-0.21	2.78

3.3.4 Antioxidant activity

Usually plant cells respond to abiotic stresses by synthesizing secondary metabolites that may protect them against the causal agent. Even though the thermal stress can be applied to increase or induce the development of secondary metabolites such as phenolic compounds in plants, there are some limitations on the dosage of the thermal stress that can be tolerated by the plant. Over dosage of thermal stress can produce sever irreversible damages to the plant or fruits. Therefore, the carrots are subjected to study the effect of PL treatment on the antioxidant activity.

Table 3.12 represents the percentage antioxidant activity calculated using Oxygen Radical Absorbance Capacity (ORAC) values for the whole treated and whole untreated carrots.

Table 3.12 %AA from ORAC values of treated and untreated whole carrots

Storage Temperature	Whole Carrot Type	Week 0	Week 1	Week 2	Week 3	Week 4
Room Temperature	Untreated[a1]	68 (± 0.3)	66 (± 0.2)	66 (± 0.3)	65 (± 0.4)	64 (± 0.3)
	Treated[a2]	86 (± 0.3)	85 (± 0.3)	84 (± 0.2)	83 (± 0.4)	82 (± 0.3)
10-15 °C	Untreated[b1]	69 (± 0.2)	68 (± 0.3)	68 (± 0.2)	67 (± 0.3)	65 (± 0.4)
	Treated[b2]	86 (± 0.3)	85 (± 0.2)	85 (± 0.5)	84 (± 0.4)	83 (± 0.3)
0-4 °C	Untreated[c1]	69 (± 0.4)	69 (± 0.2)	68 (± 0.3)	68 (± 0.5)	67 (± 0.3)
	Treated[c2]	87 (± 0.2)	86 (± 0.3)	85 (± 0.3)	85 (± 0.2)	84 (± 0.3)

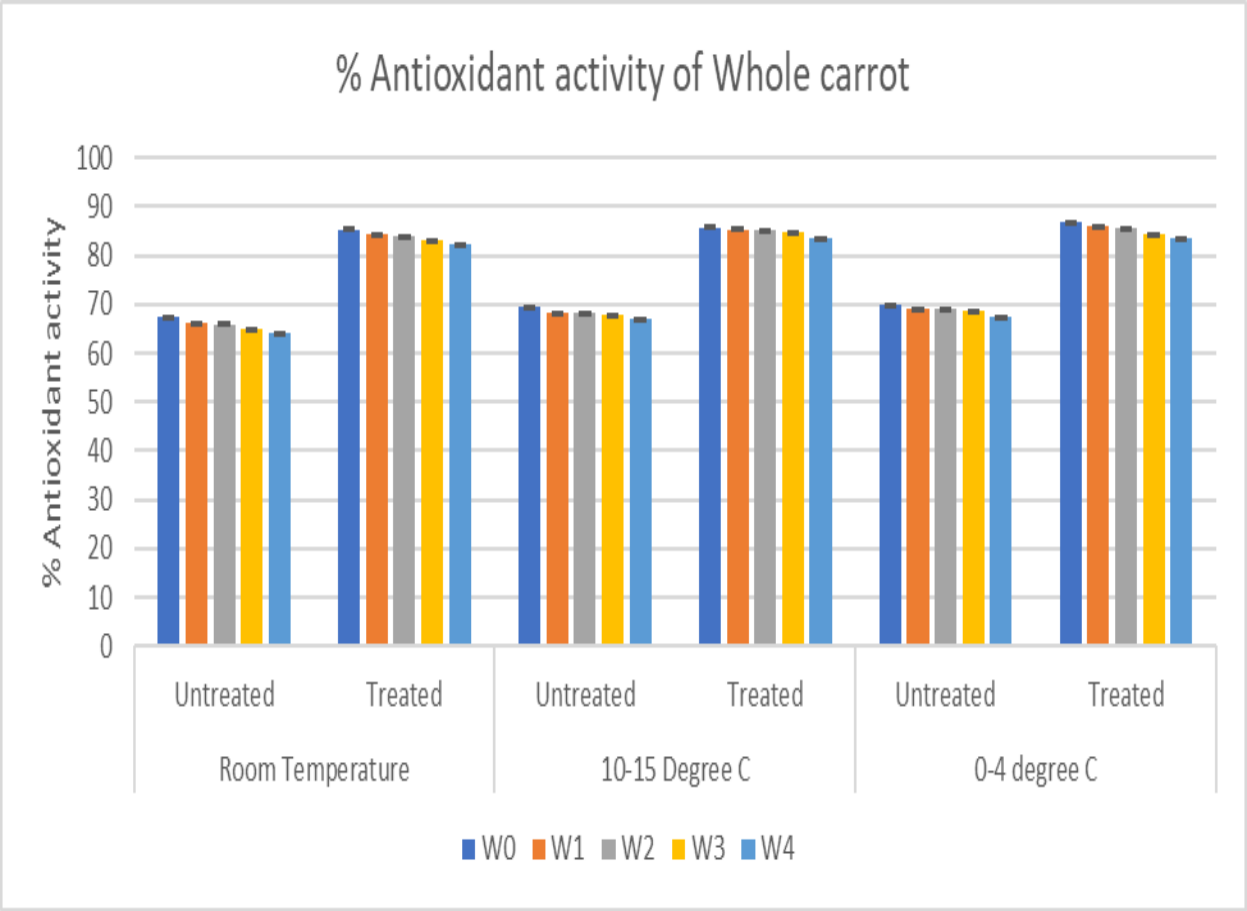


Figure 3.8 % Antioxidant activity in whole carrot

The results showed an increase of 85% in antioxidant activity after treatment with PL at 11.25 J/cm^2 at storage environment for whole carrots. This increase in antioxidant activity was due to PL treatment induced abiotic stress in the plant cells, and the stress had stimulated the synthesis of various secondary metabolites such as phenolic compounds.

Table 3.13 t-Test: Two-sample assuming equal or unequal variances for whole carrots

Storage	Whole Carrot Types	Variance (F test)	t-stat	t Critical two-tail
Room temperature	a1-a2 (s)	Equal	18.77	2.31
10-15 °C	b1-b2 (s)	Equal	-20.27	2.31
0-4 °C	c1-c2 (s)	Equal	27.20	2.31

Table 3.14 %AA from ORAC values of treated and untreated cut carrots

Storage Temperature	Cut Carrot Type	Week 0	Week 1	Week 2
Room Temperature	Untreated [a1]	67 (± 0.3)	62 (± 0.2)	60 (± 0.3)
	Treated 1 [a2]	88 (± 0.3)	87 (± 0.4)	81 (± 0.2)
	Treated 2 [a3]	90 (± 0.2)	88 (± 0.3)	82 (± 0.3)
10-15 °C	Untreated [b1]	70 (± 0.4)	69 (± 0.3)	65 (± 0.3)
	Treated 1 [b2]	89 (± 0.3)	88 (± 0.2)	82 (± 0.3)
	Treated 2 [b3]	90 (± 0.3)	90 (± 0.4)	83 (± 0.3)
0-4 °C	Untreated [c1]	67 (± 0.2)	67 (± 0.3)	66 (± 0.3)
	Treated 1 [c2]	89 (± 0.3)	89 (± 0.3)	84 (± 0.4)
	Treated 2 [c3]	91 (± 0.3)	90 (± 0.2)	85 (± 0.2)

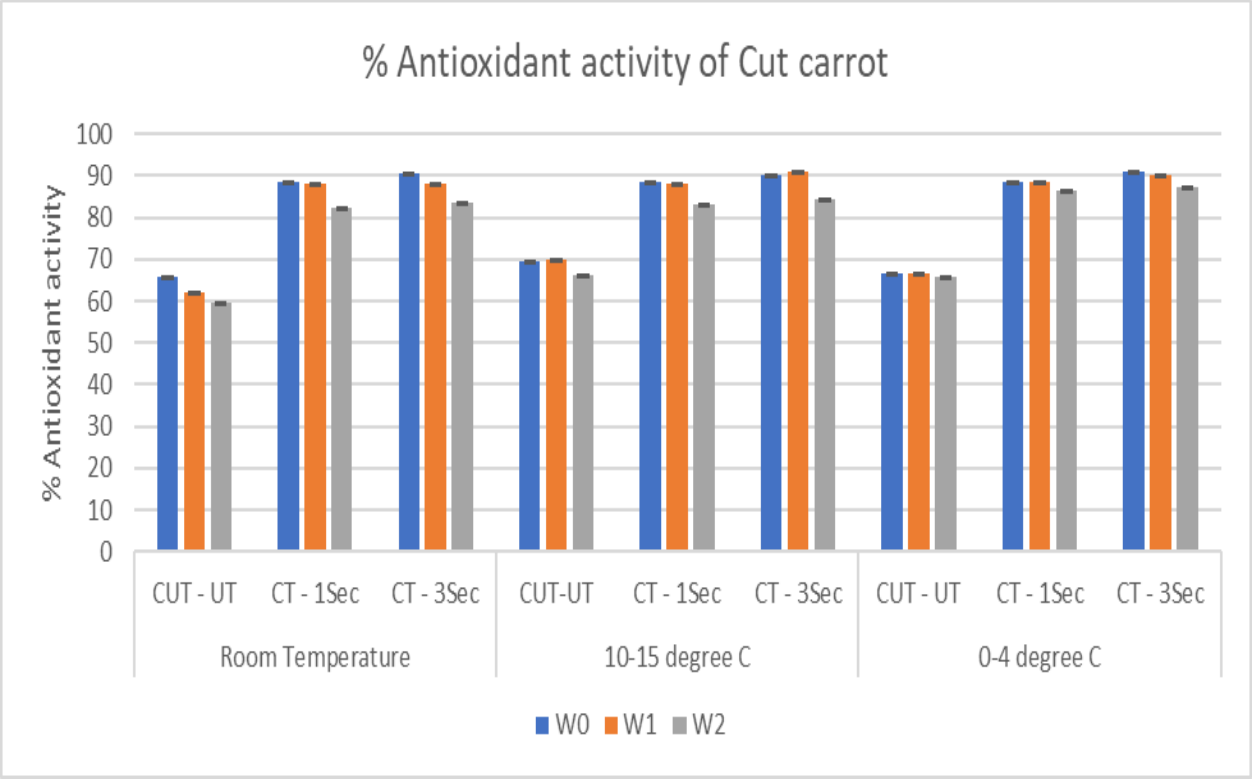


Figure 3.9 % Antioxidant activity of cut carrots

Whereas, the cut carrots post PL treatments for 1 s and 3 s showed 89% and 91% increase in antioxidant activity. This might be due to greater stress generated in cut carrot tissues treated with PL of 3 s compared to 1 s. Therefore, the designed dosage of PL treatment showed a positive impact on the quality of whole and cut carrots at appropriate storage temperatures.

Table 3.15 t-Test: Two-sample assuming equal or unequal variances for cut carrots

Storage	Cut Carrot types	Variance (F test)	t-stat	t Critical two-tail
Room temperature	a1-a2 (s)	Equal	7.40	2.78
	a1-a3 (s)	Equal	7.44	2.78
10-15 °C	b1-b2 (s)	Equal	6.88	2.78
	b1-b3 (s)	Equal	7.05	2.78
0-4 °C	c1-c2 (s)	Unequal	12.16	4.30
	c1-c3 (s)	Unequal	11.67	4.30

3.3.5 Peroxidase activity

Peroxidases are found in almost all vegetables. The activity of these enzymes can be an indicator of the vegetable quality as it normally increases during ripening (Prestamo G. & Manzano P., 1993).

The peroxidase enzyme did not observe to get deactivated post treatment with PL at experimented energy level and continued to remain active throughout the shelf life at the experimented storage conditions.

3.3.6 Microbiological study of aerobic and yeast & mold count with storage time

Applications of PL for microbial inactivation are limited to the product's surfaces, most studies have tested the effectiveness of PL on food or packaging surfaces. For instance, the process was reported to be effective to inactivate molds in a variety of baked goods and to extend their shelf lives (Dunn et al., 1995). Apart from that, Scott (2003) reported that consumption of contaminated food leads to illness and several food-borne diseases that remain major health problems throughout the world. Thus, pulsed light treatment is subjected in this study as a non-thermal decontamination technology for food products to achieve effective inactivation of micro-organisms.

Table 3.16 represents the log reduction in total aerobic count for the whole treated and whole untreated carrots.

Table 3.16 Total aerobic log reduction in treated with untreated whole carrots

Storage Temperature	**Whole Carrot Type**	**Week 0**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Room Temperature	Untreated[a1]	4.76 (±0.14)	5.10 (±0.21)	5.68 (±0.18)	6.51 (±0.20)	6.92 (±0.15)
	Treated[a2]	4.55 (±0.18)	4.86 (±0.13)	4.60 (0.21)	6.42 (±0.19)	6.89 (±0.18)
10-15 °C	Untreated[b1]	4.76 (±0.14)	4.15 (±0.23)	5.46 (±0.17)	6.33 (±0.14)	6.85 (±0.08)
	Treated[b2]	4.55 (±0.23)	4.63 (±0.15)	5.32 (±0.18)	6.40 (±0.05)	6.87 (±0.12)
0-4 °C	Untreated[c1]	4.76 (±0.15)	5.08 (±0.18)	5.27 (±0.14)	6.53 (±0.17)	6.95 (±0.10)
	Treated[c2]	4.55 (±0.22)	4.33 (±0.22)	5.03 (±0.09)	6.56 (±0.18)	6.98 (±0.12)

The aerobic count increased during storage at room temperature with highest growth seen at the end of 3rd week. Similar growth pattern was observed at refrigerated storage conditions on both treated and untreated whole carrots.

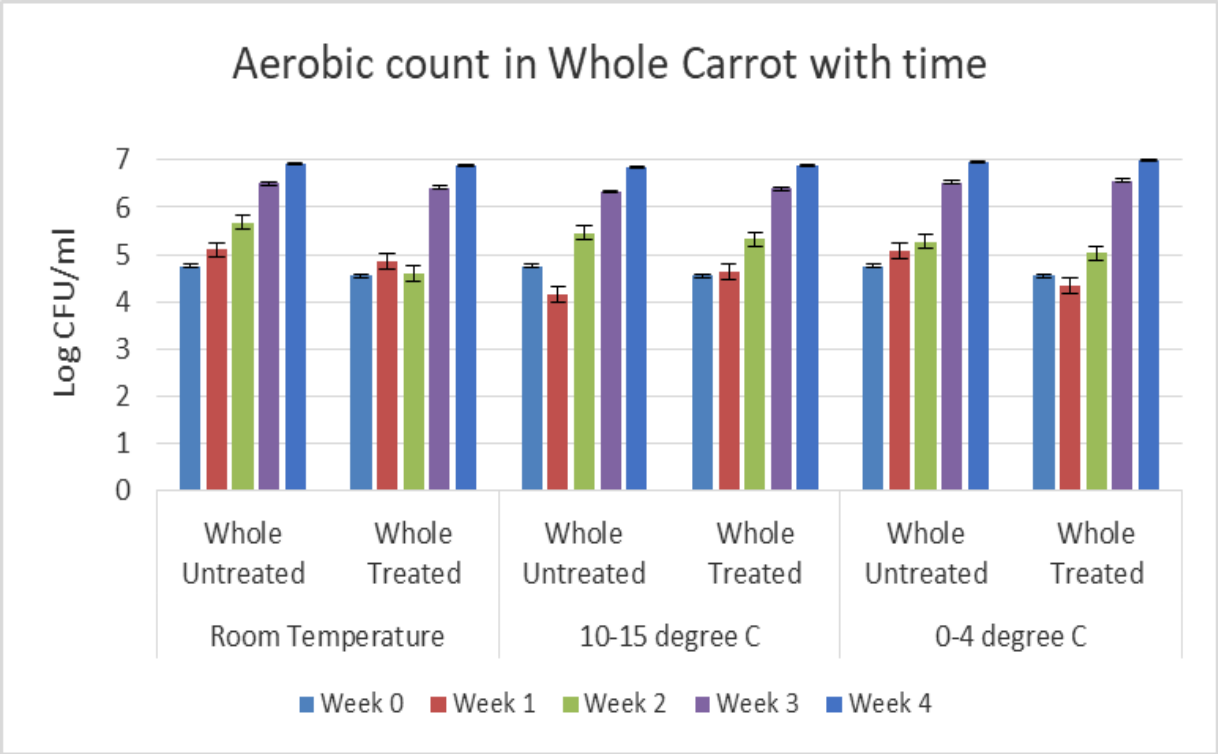


Figure 3.10 Comparison of aerobic count log reduction for whole carrot

The result showed treatment showed log reduction of 0.21 in aerobic count whole carrots. Thus, PLT showed positive impact as a non-thermal decontamination technology for whole carrots to achieve effective log reduction of total aerobic count. The statistical study result is depicted in the Table 3.17 for whole carrot.

Table 3.17 t-Test: Two-sample assuming equal or unequal variances for whole carrots

Storage	Whole Carrot types	Variance (F test)	t-stat	t Critical two-tail
Room temperature	a1-a2 (ns)	Equal	-0.51	2.31
10-15 °C	b1-b2 (ns)	Equal	-0.06	2.31
0-4 °C	c1-c2 (ns)	Equal	-0.33	2.31

The comparative study was conducted to verify the effectiveness of PLT on the cut carrots. The study results are demonstrated in Figure 3.12.

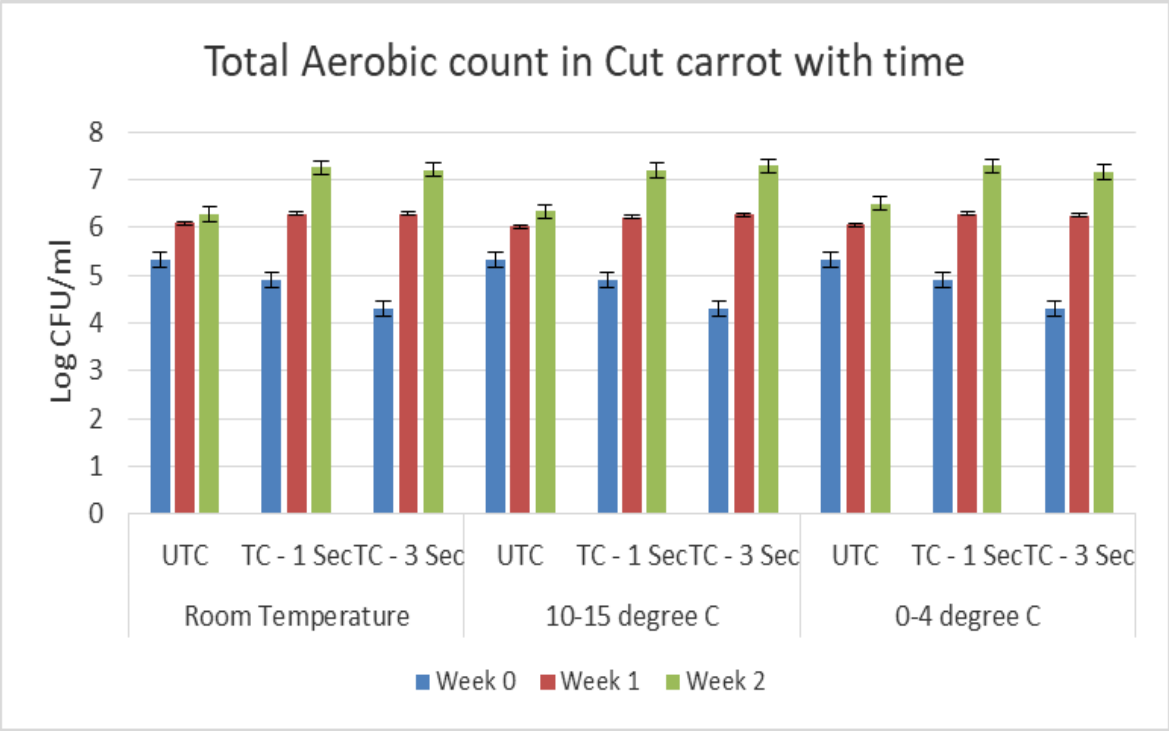


Figure 3.11 Comparison of Aerobic count in log reduction for cut carrot

Treatment time of 1 second showed log reduction as 0.42, while the treatment time of 3 seconds showed 1.02 log reduction in cut carrots. Higher log reduction is achieved by increasing the treatment time. Contrarily, increasing treatment time rapid in spoilage for cut carrots due to an increase in abiotic stress. The statistical study result is depicted in the Table 3.18 for cut carrot.

Table 3.18 t-Test: Two-sample assuming equal or unequal variances for cut carrots

Storage	Cut Carrot types	Variance (F test)	t-stat	t Critical two-tail
Room temperature	a1-a2 (ns)	Equal	0.33	2.78
	a1-a3 (ns)	Equal	0.04	2.78
10-15 °C	b1-b2 (ns)	Equal	0.29	2.78
	b1-b3 (ns)	Equal	0.06	2.78
0-4 °C	c1-c2 (ns)	Equal	0.25	2.78
	c1-c3 (ns)	Equal	-0.06	2.78

Shelf life study supported increase the count of yeast and molds with longer storage time due to presence of favorable growth conditions such as free moisture, nutrients, etc. Table 3.19 represents the yeast and mold log reduction of the whole treated and whole untreated carrots.

Table 3.19 Yeast and mold log reduction in treated with untreated whole carrots

Storage Temperature	**Whole Carrot Type**	**Week 0**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Room Temperature	Untreated[a1]	2.88 (±0.14)	4.74 (±0.21)	4.98 (±0.18)	5.28 (±0.20)	6.35 (±0.15)
	Treated[a2]	2.76 (±0.18)	4.28 (±0.13)	5.03 (0.21)	5.48 (±0.19)	6.73 (±0.18)
10-15 °C	Untreated[b1]	2.88 (±0.14)	4.02 (±0.23)	4.81 (±0.17)	5.20 (±0.14)	6.44 (±0.08)
	Treated[b2]	2.76 (±0.23)	3.79 (±0.15)	4.23 (±0.18)	5.02 (±0.05)	6.62 (±0.12)
0-4 °C	Untreated[c1]	2.88 (±0.15)	3.73 (±0.18)	5.28 (±0.14)	5.59 (±0.17)	6.51 (±0.10)
	Treated[c2]	2.76 (±0.22)	3.72 (±0.22)	4.08 (±0.09)	5.12 (±0.18)	6.59 (±0.12)

The results showed only 0.12 log reduction with 11.25 J/cm^2 energy of PL in case of whole carrots. The graphical representation of the result is shown in Figure 3.13.

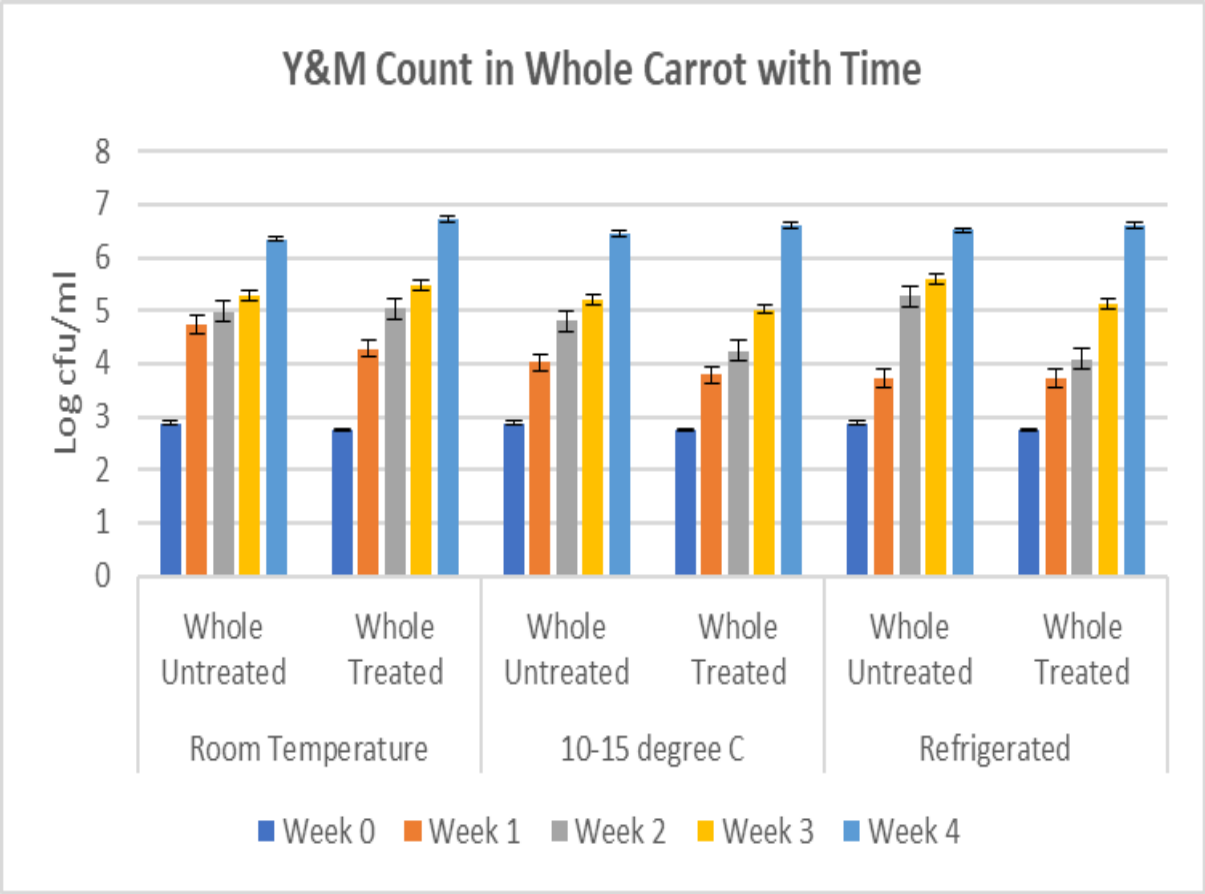


Figure 3.12 Comparison of yeast & mold count in log reduction for whole carrot

The following Table 3.20 has depicted statistical study result for whole carrot.

Table 3.20 t-Test: Two-sample assuming equal or unequal variances for whole carrots

Storage	Whole Carrot types	Variance (F test)	t-stat	t Critical two-tail
Room temperature	a1-a2 (ns)	Equal	0.01	2.31
10-15 °C	b1-b2 (ns)	Equal	-0.21	2.31
0-4 °C	c1-c2 (ns)	Equal	0.37	2.31

The graphical representation of the study result for cut carrots is shown in Figure 3.15 for log reduction in yeast and mold count.

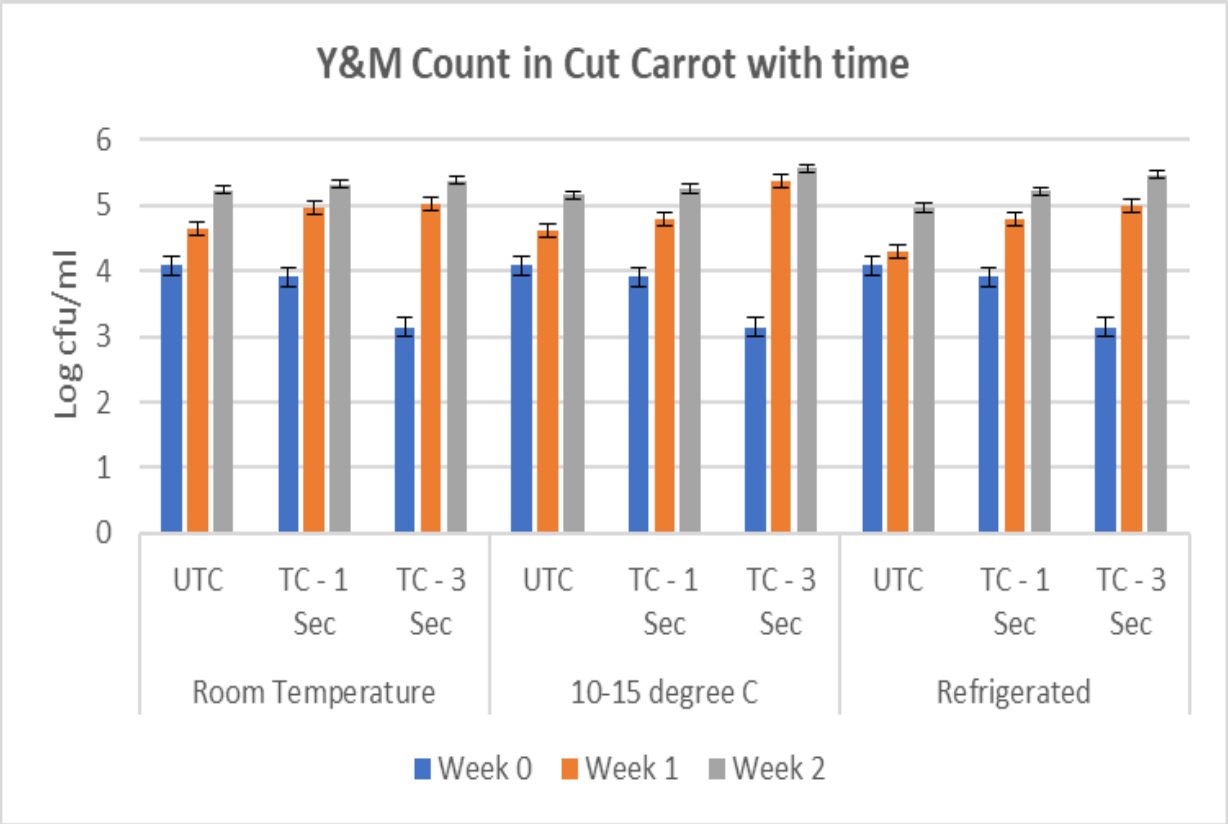


Figure 3.13 Comparison of yeast & mold count in log reduction for cut carrot

The study revealed that higher log reduction in yeast and mold counts with increase in energy of PL treatment in case of cut carrots. This could be supported by the result as PL treatment at 2.25 J/cm^2 and 6.75 J/cm^2 energy resulted in 0.17 and 0.94 log reduction in Yeast and Mold counts. The statistical study results are summarized in the Table 3.17 for cut carrot.

Table 3.21 t-Test: Two-sample assuming equal or unequal variances for cut carrots

Storage	Cut Carrot types	Variance (F test)	t-stat	t Critical two-tail
Room temperature	a1-a2 (ns)	Equal	0.13	2.78
	a1-a3 (ns)	Equal	-0.15	2.78
10-15 °C	b1-b2 (ns)	Equal	0.11	2.78
	b1-b3 (ns)	Equal	-0.02	2.78
0-4 °C	c1-c2 (ns)	Equal	0.42	2.78
	c1-c3 (ns)	Equal	0.05	2.78

3.4 Conclusions

The effect of PLT was found significant in the quality in terms of Antioxidant and microbial log reduction of the carrots compared to untreated carrots. However, the treatment showed minimal changes in the overall shelf life of the whole carrots, while negatively impacted on the shelf life of cut carrots due to PL treatment caused damage to the exposed plant cells.

PL treatment caused a lower rate of change in pH with storage time for whole carrots. Contrary, it increases the moisture loss towards the end of the shelf-life. Further post-treatment with PL, the peroxidase enzyme did not get deactivated at the experimented energy level.

Moreover, PLT enhanced the antioxidant properties of carrots by increasing their total polyphenolic content. This illustrates the potential use of pulsed UV light to enhance the nutritional content of carrots. PLT increased greater antioxidant activity for cut carrots compared to whole carrots. The antioxidant activity of whole carrots showed an increase of 85% after treatment with PL at 11.25 J/cm^2 at the storage environment. Whereas, the cut carrots after treatment to PL treatments of 1 s and 3 s showed 89% and 91% increase in antioxidant activity.

The result showed total microflora log reduction in terms of aerobic count and yeast and mold count post the treatment. Hence, a PL treatment found its usefulness as a novel, non-thermal surface microflora decontamination technology for food products to achieve effective inactivation of micro-organisms.

In conclusion, this study showed usefulness in investigating the treatment for achieving the desired quality in carrots.

PREFACE TO CHAPTER 4

Application of UV light for microbial inactivation has been in use in different sectors since many years because of the bactericidal effect of UV light. In the previous chapter, the effect of PL treatment provided at different doses on whole and cut carrots were studied. This chapter focuses on the shelf life and quality enhancement study in comparison to PL treatment versus UV light treatment on fresh radish (whole). PL treatment doses were selected within the FDA approved limit of application on food surfaces and corresponding UV light treatment doses were also determined for application on fresh radish. Samples from both treatments were stored at different temperatures and the shelf life was monitored for 28 days.

The combined results of Chapter 3 and Chapter 4 helps to determine the effectiveness of both these surface decontamination techniques for application as a post-harvest microbial intervention method on foods as well as to study the effect of these treatments on shelf-life and quality, in terms of nutritional parameters, of the product. The results obtained could prove beneficial for knowledge towards designing PL equipment suitable for commercial applications either for farm use or for different food industries.

All experimental work and data analysis were conducted by the candidate under supervision of Dr. H. S. Ramaswamy.

Prusty, P., Ramaswamy, H.S., 2019. Effectiveness of Pulsed Light (PL) and UV-C light treatments on the quality and shelf-life of fresh carrot and radish. Poster presented at CTAQ meeting June 2018 (draft).

CHAPTER 4

COMPARATIVE STUDY OF PULSED AND UV-C LIGHT TREATMENT ON SHELF LIFE AND QUALITY ENHANCEMENT OF FRESH RADISH

Abstract

In this work, washed, air-dried and sorted radish were exposed to different doses of PL (3 pulses/1 sec, 6 pulses/2 sec and 9 pulses/3 sec) for comparative study the effect with UV-C irradiation (2.25 J/cm^2, 4.5 J/cm^2 and 6.75 J/cm^2). Later it stored at room temperature, 10-15 °C and 0-4 °C for up to 28 days. The effects of light treatments on the physical-chemical properties and antioxidant compounds of radish were evaluated during storage (at week 0, week 1, week 2, week 3 and week 4) and comparative study was carried out with those of untreated samples. The results demonstrated that pH and moisture loss of all the samples were not affected by both light treatments (PL and UV-C) at the energy investigated dosage. The skin color of untreated and treated radish turned from shining red to dark black during storage at room temperature, and the light treatments (PL and UV-C) at the highest dosage was responsible for faster in the rate of deterioration in the case of radish. Moreover, this study has shown the positive impact of PL treatment over the UV-C treatment on the quality of the whole radish at an appropriate storage temperature in terms of antioxidant activity. Besides, the higher log reduction was achieved for yeast and mold count with the increase in energy of UV-C and PL doses. The result of PL treated radish with 2.25 J/cm^2, 4.5 J/cm^2, and 6.75 J/cm^2 energy showed log reduction in yeast and mold counts were 0.46, 0.79, and 0.38 at room temperature, 10-15 °C, and 0-4 °C respectively. Whereas, UV-C treated radish demonstrated the 0.65, 0.91, and 0.71 log reduction with equivalent energy level of PL. Thus, the overall study results concluded that PL treatment is better compared to UV-C irradiation due to its' potential to enhance the accumulation of health-beneficial food compounds in radish without significant changes in the physical properties of the product during storage.

4.1 Introduction

The market of fresh-cut fruits and vegetables (F&V) (washed, cut, packaged and refrigerated products) is the fastest growing segment. However, minimal processing operations damage F&V tissue integrity which triggers deteriorative processes including oxidative browning, tissue softening, water loss and development of undesirable flavors and odors (Mastromatteo et al., 2009).

UV light radiation has been used for many years in pharmaceutical, electronic, and aquaculture industries. The use of UV light for food disinfection has been wrongly associated with loss of nutritional value and undesirable appearance, which may be true when using very high UV doses (J.A. Guerrero-Beltrán and G.V. Barbosa-Cánovas*, 2004).

Pulsed light is a new method intended for the decontamination of food surfaces using short, high frequency pulses of an intense broad spectrum. This technology has potential applications for the treatment of foods, packaging and processing equipment for the food, medical and pharmaceutical industries, water and air. Additionally, the use of pulsed UV light has proven effective for the inactivation of test species on surfaces and suspension at a more rapid rate than standard UV methods (Garvey et al., 2014).

Therefore, the research was carried out to compare the effectiveness of the treatment, which allow the radish not only to last longer but also to improve their quality and nutritional benefits.

4.2 Materials and Methods

4.2.1 Materials

Radish from local grocery store were obtained on the day of treatments with UV light and PL for the experiment. The radish were washed and air dried prior to use. Damaged or blemished radish were sorted out.

(a) Whole (b) Trimmed, washed and sorted

Figure 4.1 Fresh Radish

4.2.2 Research Methods

4.2.2.1 Treatment of pulsed light

PL is produced using a R&D Benchtop pulsed light system - Steripulse-XL (RS-3000C, Xenon Corp., Wilmington, Mass., U.S.A.). The sample was placed at the center of a glass container (covered with aluminum foil for maximum light reflection onto the sample) and further it has been subjected to the following treatment times: 1 pulse, 2 pulses and 4 pulses on each side of the sample.

Steripulse–XL PL instrument is a high energy system used for decontamination of food, medical devices, packaging materials, blood or pharmaceutical products. The equipment is equipped with pulsed UV/visible lamps capable of delivering 3 pulses per second of 1.27 Joules/cm^2/pulse. Excessive heat buildup is prevented by using very short pulse (360 μs width). Since the lamp is made of Xenon, there is no hazardous risk as compared to mercury lamp in the traditional UV lamps. The treatment chamber has forced air evacuation mechanism to ensure no heat buildup from the lamp. The forced air has filters capable of filtering microbe and ozone resistant at both the ends. The chamber has interlocking mechanism with a safety interlock switch to prevent undesirable exposure to the operator. The chamber is made of stainless steel for easy sterilization and cleaning before and after each treatment (Figure 4.2).

Figure 4.2 Radish treated with Pulsed Light

The sterilization system is connected with a controller to control the pulse application. The pulse rate is 3 pulses/second and pulse mode can be either timed or continuous. The

controller has a programmable timer with a range from 1 to 999 seconds in 1 second interval. The sterilization chamber has racks at different heights and the energy exposure level varies depending on the location inside the chamber (Figure 4.2).

4.2.2.2 UV light treatment

The UV treatment of samples were carried out in Air Science UV-Box. Treatment times were calculated corresponding to the energies subjected to the samples by PL treatment. The treatment was provided on each side of the sample like the PL treatment provided for carrots in the previous chapter.

The UV-Box is (manufactured by Air Science) specially designed for highly efficient and safe decontamination of microbes. The equipment is equipped with 254nm UV lamps, optimally placed in the chamber to eliminate any blind spots. The UV lamps is capable of emitting 52.5 watts of UC-C radiation. Corners and walls are made of Stainless-Steel surfaces for natural reflection of radiation energy. Adjustable UV timer helps with decontamination time and cycle. The design of the chamber is UV absorbent which protect the operators from any radiation risk (Figure 4.4). The safety feature on the chamber lock allows the lamp to activate only after the safety lock is activated. This equipment can provide UV-C radiation and the treatment energy is calculated from the emitting energy with the exposure time.

Figure 4.3 Radish treated with UV Light

4.2.3 Experimental design

The experiment has designed with 2 different factors, which are storage and treatment. Each factor has further divided into 3 levels. As part of designing the experiment, different level of energy application had been designed and applied based on the FDA guidelines (CFR - Code of Federal Regulations Title 21) for the maximum exposure level by using the pulsed light and UV light systems. Fresh whole radish were treated with PL fluences of 2.25, 4.5 and 6.75 J/cm^2 and corresponding UV-C light doses according to design and compared with a control set without treatment. Samples were stored at three different storage conditions (Room temperature, Refrigerated 10-15 °C and 0-4 °C).

The following Table 4.1 depicts the treatment information of pulsed light and UV-C light as well as studied storage conditions for radish:

Table 4.1 Experimental design set point for treatment of radish

Radish	Pulsed Light Treatment (P)	UV-C light Treatment (C)	Storage
Fresh Whole	2.25 J/cm^2 **or** (3 pulses / 1 sec), 4.5 J/cm^2 **or** (6 pulses / 2 sec), and 6.75 J/cm^2 **or** (9 pulses / 3 sec)	2.25 J/cm^2, 4.5 J/cm^2, and 6.75 J/cm^2	Room Temperature, Refrigerated (0-4 °C and at 10-15 °C)
Control **(B)**	No treatment	No treatment	Room Temperature, Refrigerated (0-4 °C and at 10-15 °C)

Evaluation was carried out on Day 0 for appearance, moisture loss, pH, antioxidant activity, peroxidase activity, total aerobic count and Yeast & Molds to compare the qualitative changes to the treated compared with untreated. Similar quality parameters were evaluated by repeating at Day 7 (week 1), Day 14 (week 2), Day 21 (week 3), and Day 28 (week 4) to monitor the changes in quality with time at different storage conditions (Table 4.2).

Table 4.2 Experimental protocol

Experiment sample Protocol								
	Storage Temp		Pulse			UV-C		
		Blank	3 Pulse/ 1Sec	6 Pulse/ 2 Sec	9 Pulse / 3 Sec	2.1J/Cm2	4.2J/Cm2	6.3J/Cm2
Week 0	Room Temp	B1	P1-W0	P4-W0	P7-W0	C1-W0	C4-W0	C7-W0
	10-15 degree C	B2	P2-W0	P5-W0	P8-W0	C2-W0	C5-W0	C8-W0
	0-4 degree C	B3	P3-W0	P6-W0	P9-W0	C3-W0	C6-W0	C9-W0
	Storage Temp		Pulse			UV-C		
		Blank	3 Pulse/ 1Sec	6 Pulse/ 2 Sec	9 Pulse / 3 Sec	2.1J/Cm2	4.2J/Cm2	6.3J/Cm2
Week 1	Room Temp	B1-W1	P1-W1	P4-W1	P7-W1	C1-W1	C4-W1	C7-W1
	10-15 degree C	B2-W1	P2-W1	P5-W1	P8-W1	C2-W1	C5-W1	C8-W1
	0-4 degree C	B3-W1	P3-W1	P6-W1	P9-W1	C3-W1	C6-W1	C9-W1
	Storage Temp		Pulse			UV-C		
		Blank	3 Pulse/ 1Sec	6 Pulse/ 2 Sec	9 Pulse / 3 Sec	2.1J/Cm2	4.2J/Cm2	6.3J/Cm2
Week 2	Room Temp	B1-W2	P1-W2	P4-W2	P7-W2	C1-W2	C4-W2	C7-W2
	10-15 degree C	B2-W2	P2-W2	P5-W2	P8-W2	C2-W2	C5-W2	C8-W2
	0-4 degree C	B3-W2	P3-W2	P6-W2	P9-W2	C3-W2	C6-W2	C9-W2
	Storage Temp		Pulse			UV-C		
		Blank	3 Pulse/ 1Sec	6 Pulse/ 2 Sec	9 Pulse / 3 Sec	2.1J/Cm2	4.2J/Cm2	6.3J/Cm2
Week 3	Room Temp	B1-W3	P1-W3	P4-W3	P7-W3	C1-W3	C4-W3	C7-W3
	10-15 degree C	B2-W3	P2-W3	P5-W3	P8-W3	C2-W3	C5-W3	C8-W3
	0-4 degree C	B3-W3	P3-W3	P6-W3	P9-W3	C3-W3	C6-W3	C9-W3
	Storage Temp		Pulse			UV-C		
		Blank	3 Pulse/ 1Sec	6 Pulse/ 2 Sec	9 Pulse / 3 Sec	2.1J/Cm2	4.2J/Cm2	6.3J/Cm2
Week 4	Room Temp	B1-W4	P1-W4	P4-W4	P7-W4	C1-W4	C4-W4	C7-W4
	10-15 degree C	B2-W4	P2-W4	P5-W4	P8-W4	C2-W4	C5-W4	C8-W4
	0-4 degree C	B3-W4	P3-W4	P6-W4	P9-W4	C3-W4	C6-W4	C9-W4

Where;

B = Blank sample or controlled sample or untreated sample

P = Pulsed light treated sample

C = UV-C light treated sample

W = Week number during when study was carried-out

4.2.4 Instruments

The following instruments were used for the treated sample analysis:

4.2.4.1 UV-Vis Spectrophotometer

Same as in section 3.2.3.1

4.2.4.2 pH meter

Same as in section 3.2.3.2

4.2.5 Analysis

The treated sample was then subjected to following analysis parameters.

4.2.5.1 Total solids

Same as in section 3.2.4.1

4.2.5.2 pH

Same as in section 3.2.4.2

4.2.5.3 Antioxidant activity

Same as in section 3.2.4.3

4.2.5.4 Peroxidase activity

Same as in section 3.2.4.4

4.2.5.5 Microbiological analysis

Same as in section 3.2.4.5

4.2.5.6 Statistical data analysis

Same as in section 3.2.5.6

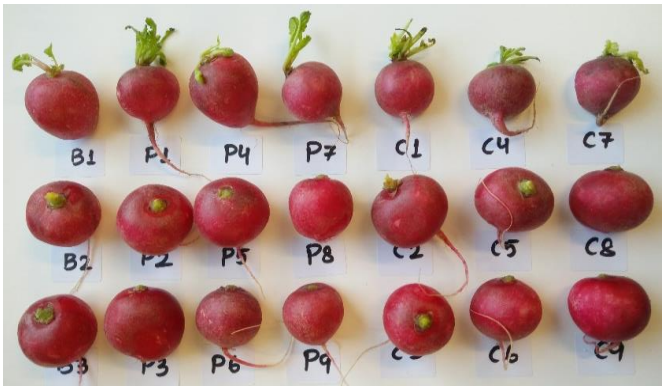
4.3 Results and Discussion

4.3.1 Effect of PL treatment on shelf-life of whole fresh radish

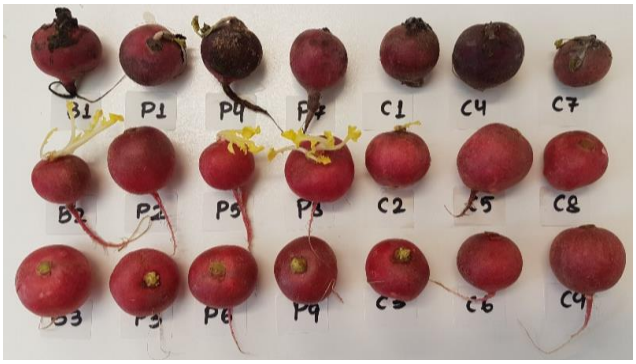
The visualization study of radish was carried out to study the effect of the treatment on overall shelf-life until the end of 4 weeks.

4.3.1.1 Physiological changes in radish with storage time

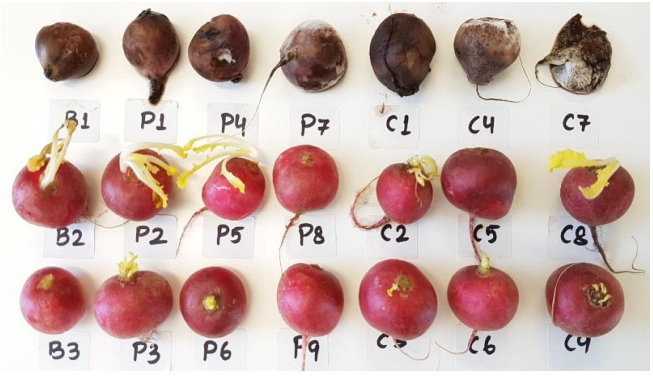
The study was concluded at the end of 3rd week for the samples stored at room temperature (B1, P1, P4, P7, C1, C4, and C7), whereas it was concluded at the end of 4th week for the refrigerated samples (B2, P2, P5, P8, C2, C5, C8, B3, P3, P6, P9, C3, C6, and C9) (Figure 4.4).



(a) Storage week 1



(b) Storage week 3



(c) Storage week 4

Figure 4.4 Physiological study observation for treated and untreated radish at different storage conditions

- Both samples (treated and untreated) stored at room temperature started to show signs of green germination at week 1; and samples got completely spoiled after end of the week 4.
- Both samples (treated and untreated) stored at 10-15 °C started to show signs of yellow germination at week 3.
- Both samples (treated and untreated) stored at 0-4 °C showed no visible differences until end of the week 4.

PL and UV-C treatments showed minimal effect on shelf-life extension compared with untreated radish stored at room temperature due to an increase in the intensity of treatment led to the rapid spoilage. However, treatments at the controlled temperatures (0-4 °C and 10-15 °C) showed a reduction in spoilage rate for this study, and so resulted in the longer shelf life of the radish.

4.3.2 pH changes with storage time

The faster changes in pH indicate the rapid chemical changes, in terms of spoilage indicator, during storage. Treated (PL and UV-C) radish were monitored to study the effect of pH changes throughout the storage period compared with the untreated control sample at three different storage temperatures. The pH values for treated and untreated radish are presented in (Figure 4.5, 4.6 and 4.7), which is ranged between 5.96 and 4.7.

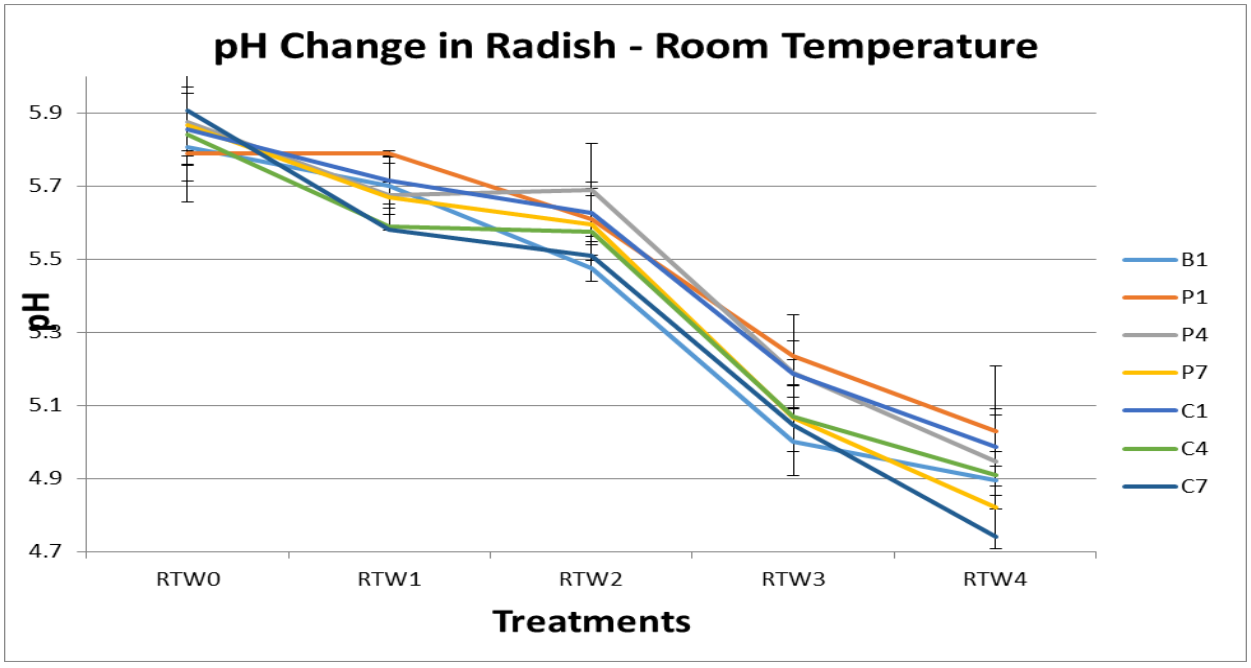


Figure 4.5 Changes in pH of Radish at room temperature

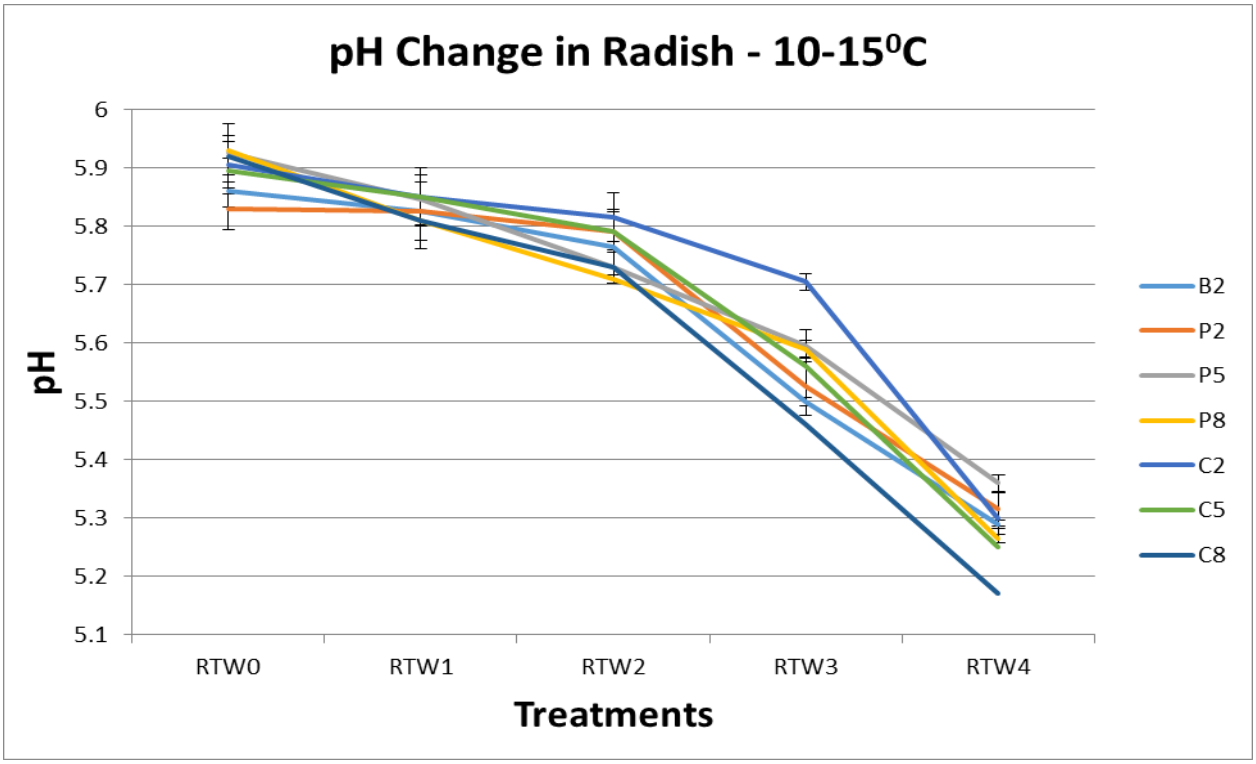


Figure 4.6 Changes in pH of Radish at 10 – 15 °C

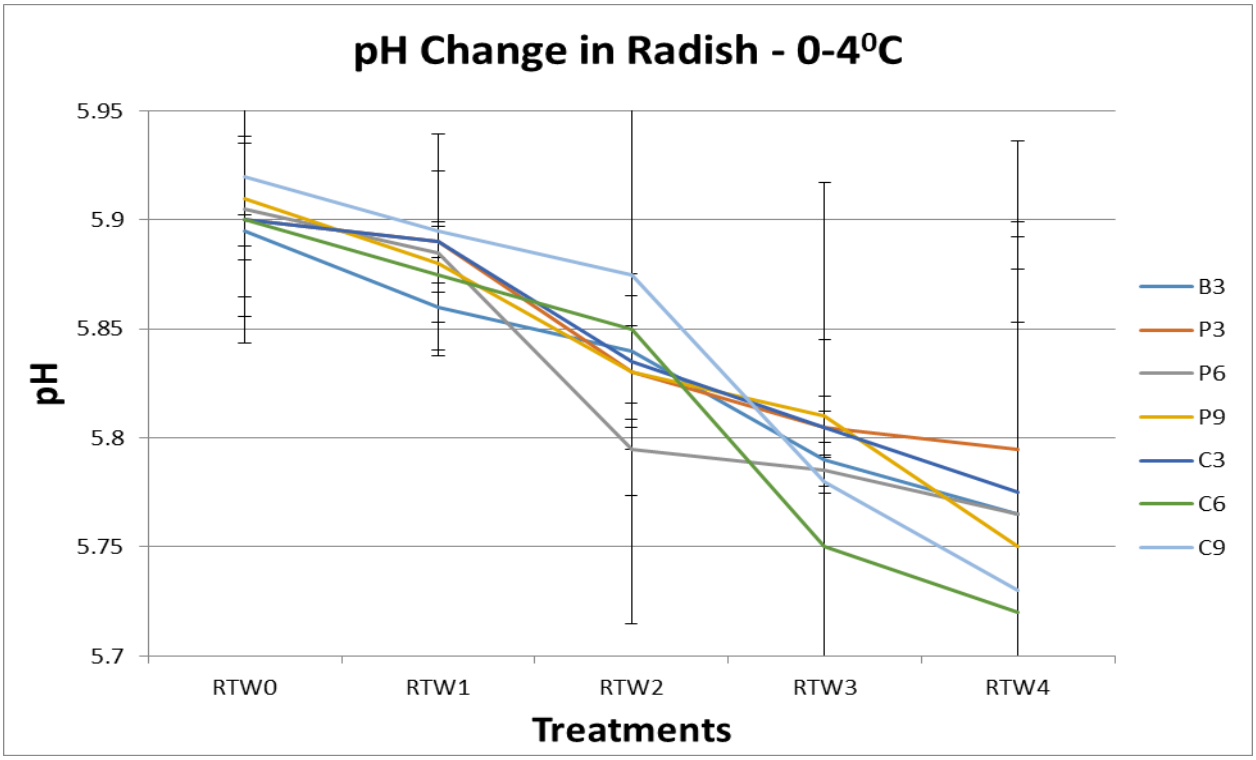


Figure 4.7 Changes in pH of Radish at 0 – 4 °C

The statistical data analysis carried-out to understand the effect of treatment on radish compared with untreated samples. The result of statistical analysis depicted in Table 4.3.

x1 = Untreated radish samples from week 0 to week 4

y1 = Pulsed light treated radish samples week 0 to week 4 (3 pulses / 1 sec)

y2 = Pulsed light treated radish samples week 0 to week 4 (6 pulses / 2 sec),

y3 = Pulsed light treated radish samples week 0 to week 4 (9 pulses / 3 sec),

z1 = UV-C light treated radish samples week 0 to week 4 (2.25 J/cm^2)

z2 = UV-C light treated radish samples week 0 to week 4 (4.5 J/cm^2)

z3 = UV-C light treated radish samples week 0 to week 4 (6.75 J/cm^2)

Table 4.3 t-Test: Two-sample assuming equal or unequal variances for radish's pH value

Storage	Radish types	Variance (F test)	t-stat	t Critical two-tail
Room temperature	x1-y1 (ns)	Equal	-0.49	2.31
	x1-z1 (ns)	Equal	-0.40	2.31
	x1-y2 (ns)	Equal	-0.40	2.31
	x1-z2 (ns)	Equal	-0.09	2.31
	x1-y3 (ns)	Equal	0.10	2.31
	x1-z3 (ns)	Equal	-0.07	2.31
10-15 °C	x1-y1 (ns)	Equal	-0.06	2.31
	x1-z1 (ns)	Equal	-0.43	2.31
	x1-y2 (ns)	Equal	-0.29	2.31
	x1-z2 (ns)	Equal	0.13	2.31
	x1-y3 (ns)	Equal	0.08	2.31
	x1-z3 (ns)	Equal	-0.17	2.31
0-4 °C	x1-y1 (ns)	Equal	-0.44	2.31
	x1-z1 (ns)	Equal	0.33	2.31
	x1-y2 (ns)	Equal	-0.08	2.31
	x1-z2 (ns)	Equal	-0.26	2.31
	x1-y3 (ns)	Equal	0.16	2.31
	x1-z3 (ns)	Equal	0.23	2.31

All samples pH values, irrespective of the treatment, eventually decreased with storage time at storage. The rate of decrease in pH was faster at room temperature, whereas the rate of decrease was slower at 10-15 °C and 0-4 °C comparatively. The study of physiological changes also supported this finding. The primary reason for this rapid pH reduction is a result of faster rate of radish spoilage particularly stored at room temperature.

In addition, the result from the comparative study shown that the rate of pH changes is lower for a radish treated with pulsed light treatment compared to the UV-C treatment. Therefore, the application of pulsed light treatment is beneficial compared to UV-C treatment within an experimented energy level in terms of maintaining the pH value of the radish in other words by slowing down the rate of spoilage.

4.3.3 Moisture loss with storage time

The faster loss in moisture indicates the rapid spoilage of the radish during storage. Treated (PL and UV-C) radish were monitored to study the effect of percentage loss in moisture throughout the storage period compared with the untreated control sample at three different storage temperatures.

The values of moisture loss in percentage for treated and untreated radish are presented in (Figures 4.8, 4.9 and 4.10), which is ranged between 0 and 30.39. The rapid moisture loss towards the end of week 3 for radish stored in room temperature storage was observed, while at a controlled temperature the rate of moisture loss observed slower.

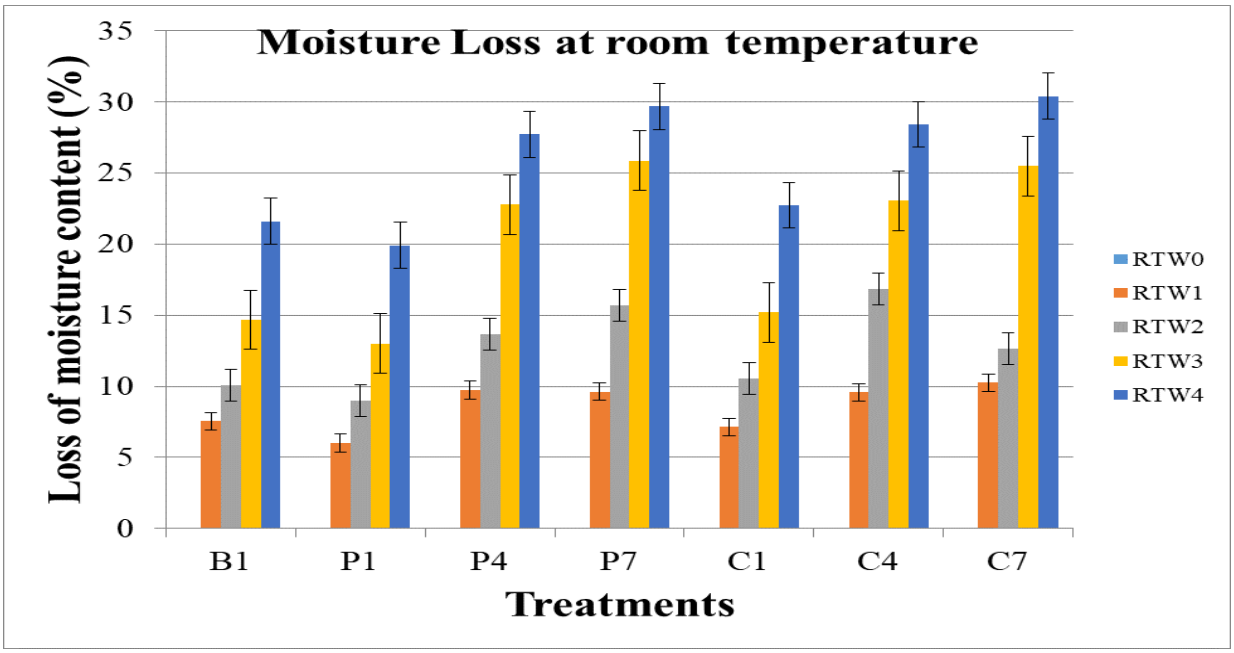


Figure 4.8 Changes in % moisture of radish at room temperature

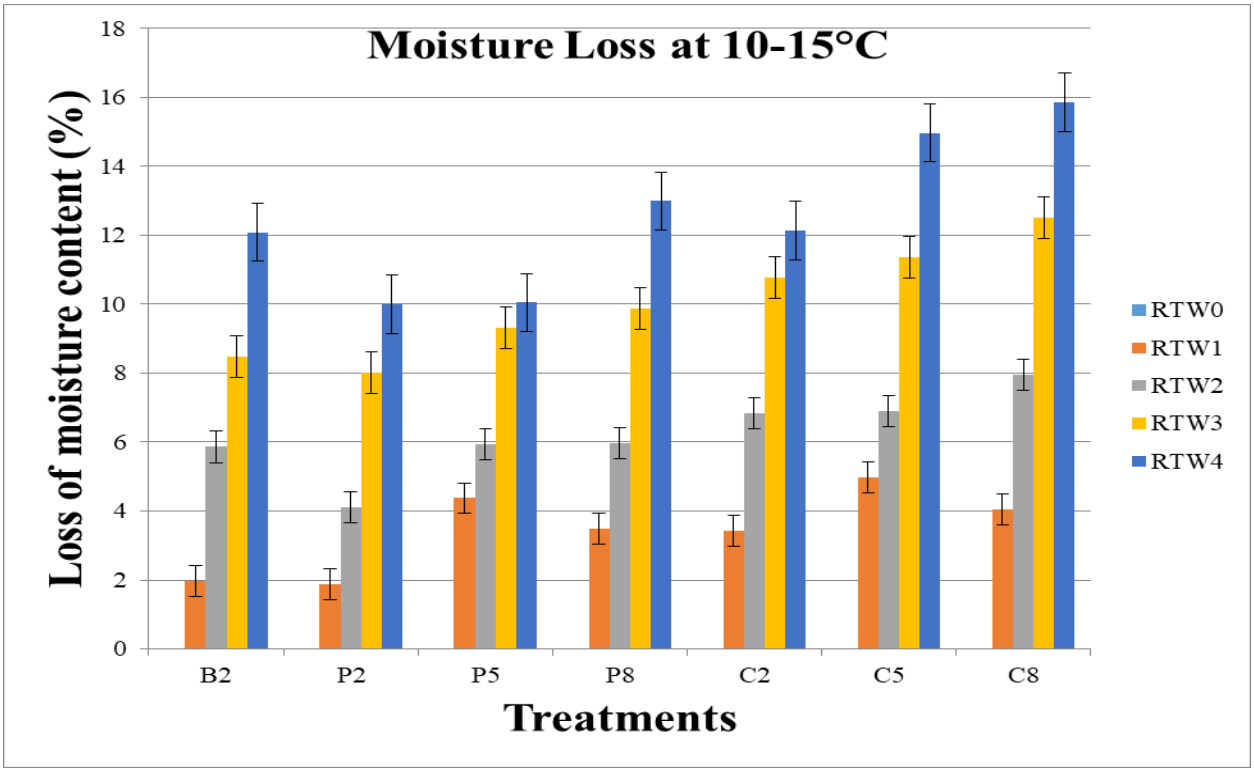


Figure 4.9 Changes in % moisture of Radish at 10 – 15 °C

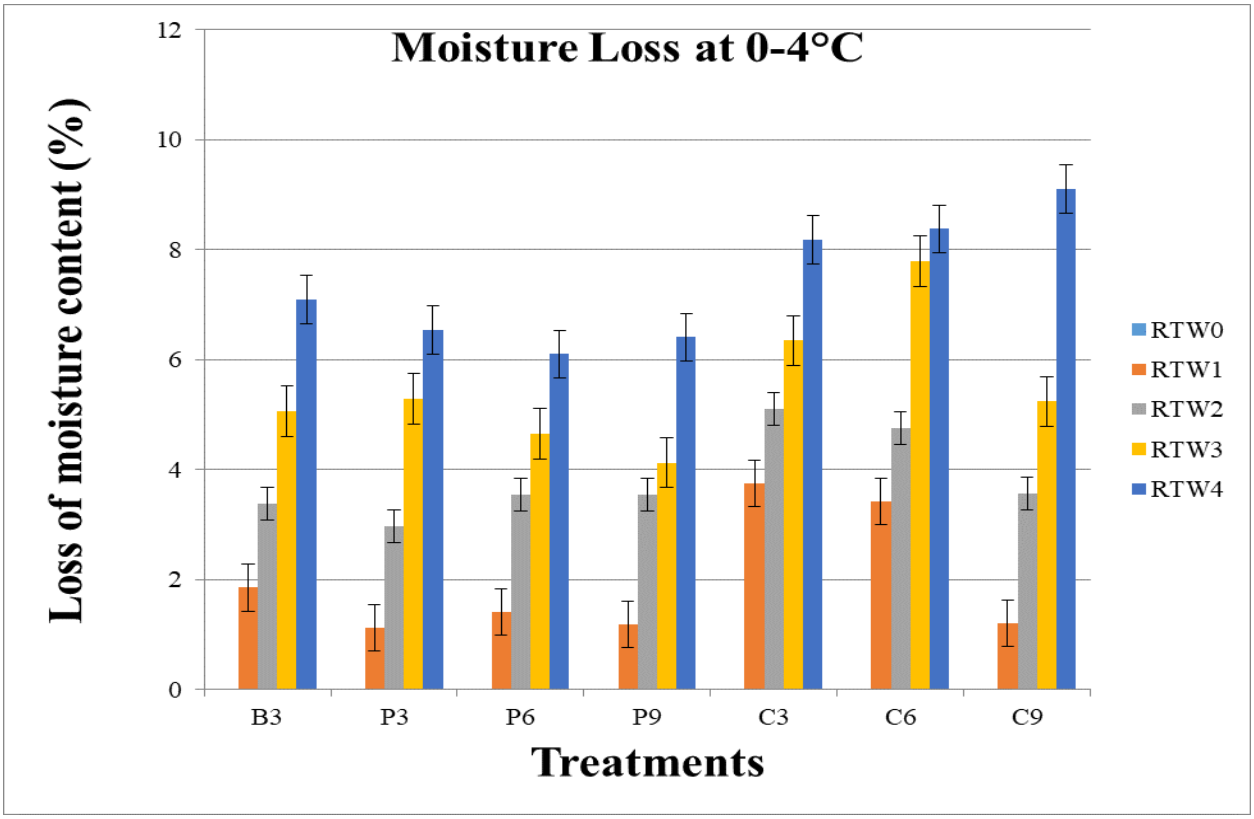


Figure 4.10 Changes in % moisture of Radish at 0 – 4 °C

The result of statistical analysis depicted in Table 4.4.

Table 4.4 t-Test: Two-sample assuming equal or unequal variances for radish's moisture loss

Storage	Radish types	Variance (F test)	t-stat	T Critical two-tail
Room temperature	x1-y1 (ns)	Equal	0.24	2.31
	x1-z1 (ns)	Equal	0.07	2.31
	x1-y2 (ns)	Equal	0.66	2.31
	x1-z2 (ns)	Equal	0.78	2.31
	x1-y3 (ns)	Equal	0.83	2.31
	x1-z3 (ns)	Equal	0.76	2.31
10-15 °C	x1-y1 (ns)	Equal	0.31	2.31
	x1-z1 (ns)	Equal	0.31	2.31
	x1-y2 (ns)	Equal	-0.09	2.31
	x1-z2 (ns)	Equal	0.58	2.31
	x1-y3 (ns)	Equal	0.25	2.31
	x1-z3 (ns)	Equal	0.67	2.31
0-4 °C	x1-y1 (ns)	Equal	0.17	2.31
	x1-z1 (ns)	Equal	0.65	2.31
	x1-y2 (ns)	Equal	0.20	2.31
	x1-z2 (ns)	Equal	0.71	2.31
	x1-y3 (ns)	Equal	0.26	2.31
	x1-z3 (ns)	Equal	0.17	2.31

Based on the results of the moisture loss with respect to time at three different storage conditions, it could be seen to have confirmed the effect of pulsed light treatments on moisture loss reduction compared to UV-C treatment. There is a gradual loss of moisture pattern was observed during the storage time. Further, the moisture loss was found higher in a treated radish compared to the untreated sample at an experimented energy level. This could have been resulted due to the effect of treatment that might have caused the damage to the outer layer of the cell's tissue. However, the treatments (PL and UV-C) did not show any positive impact on the shelf-life extension of the radish in terms of moisture loss.

4.3.4 Antioxidant activity

Antioxidants play an important role in food preservation by inhibiting oxidation processes and contributing to health promotion rendered by many dietary supplements, nutraceuticals and functional food ingredients (Shahidi F. & Zhong Y., 2015). Thermal stresses have also been demonstrated to induce the production of phenolic compounds in plants.

The changes in total polyphenolics in radish post treatment with various doses of pulsed ultraviolet rays (UV) were analyzed. The following pulsed UV durations (3 pulses/1 sec, 6 pulses/2 sec, and 9 pulses/3 sec) equivalent to three UV-C energy doses (2.25 J/cm^2, 4.5 J/cm^2, and 6.75 J/cm^2) were considered for the comparative study with respect to the untreated samples. Hence, the pulsed light treatment caused two different stresses on the radish, the stress caused by the pulsed UV light and the thermal stress created by the UV light's temperature increase on the surface of the radish.

The percentage increase or decrease in antioxidant activity of the radish is plotted in charts and shown in Figures 4.11, 4.12 and 4.13.

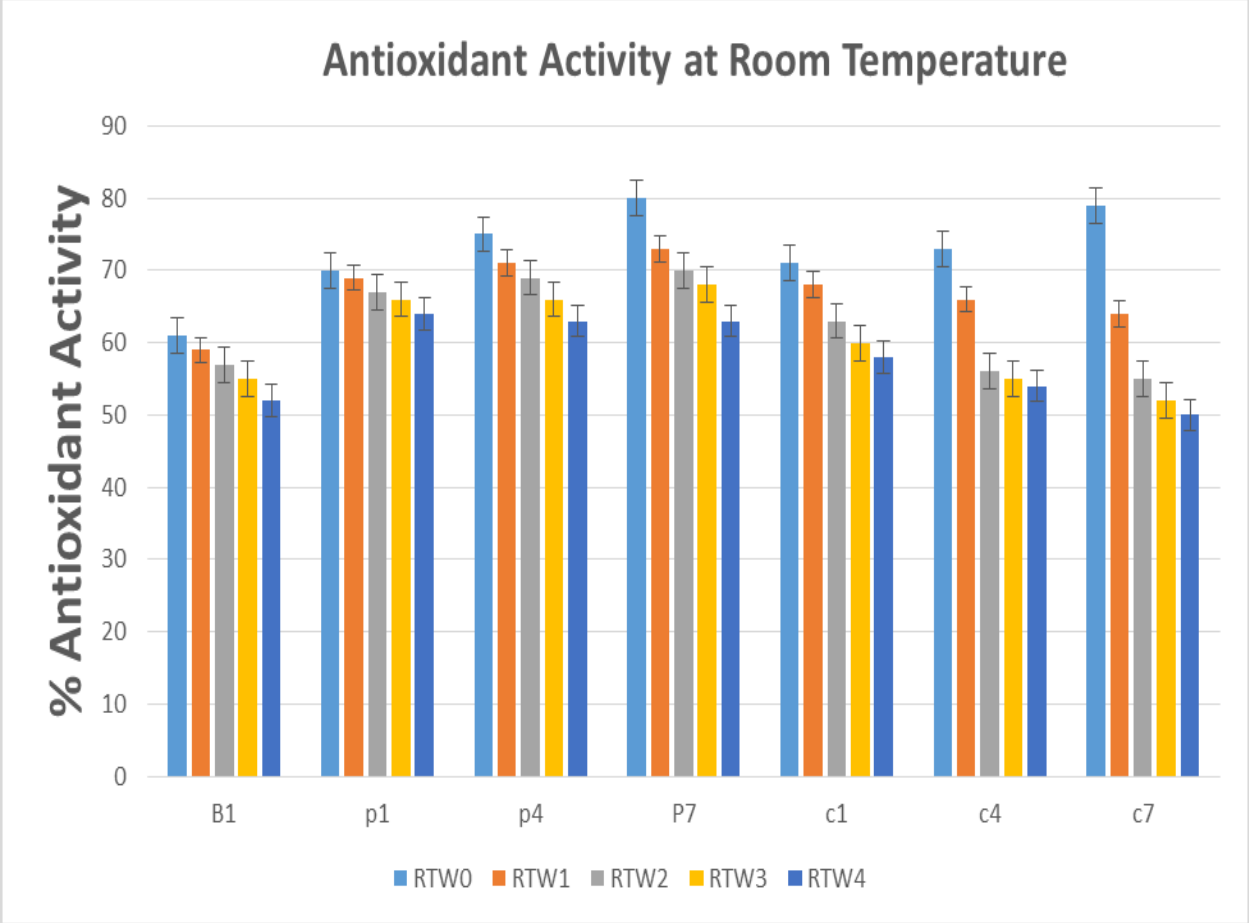


Figure 4.11 Antioxidant activity of radish at room temperature

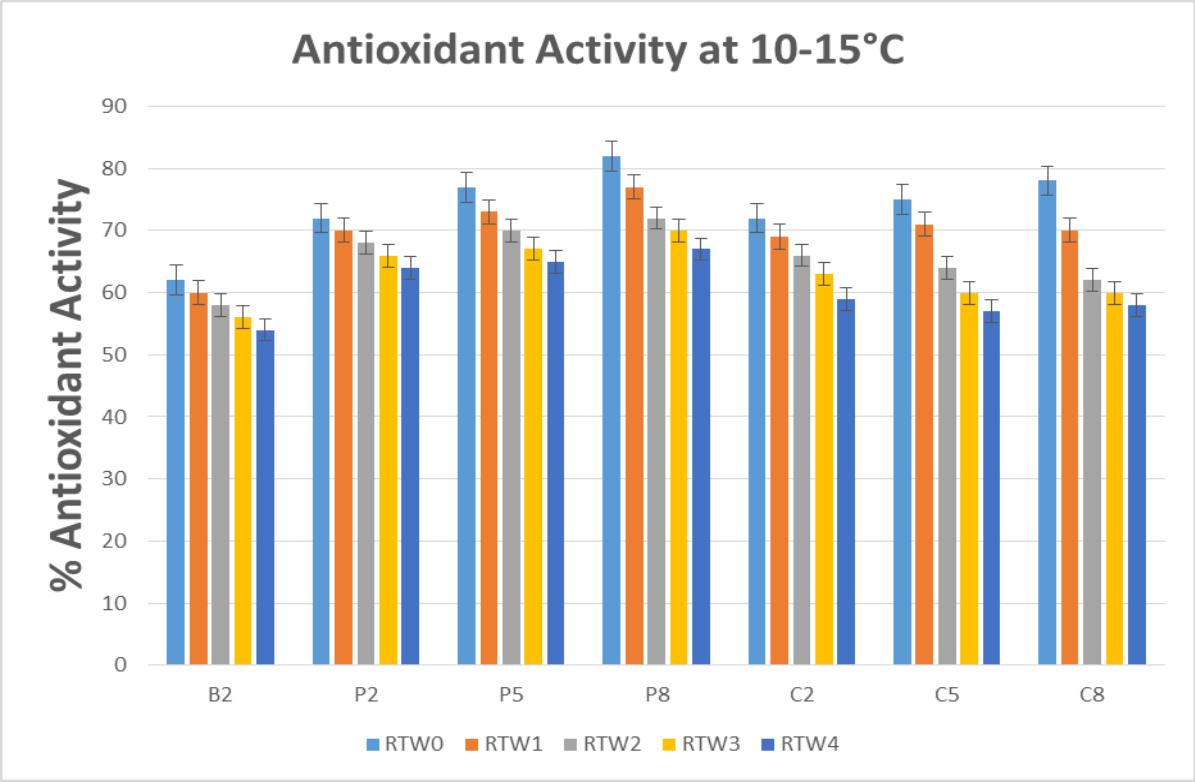


Figure 4.12 Antioxidant activity of radish at 10 – 15 °C

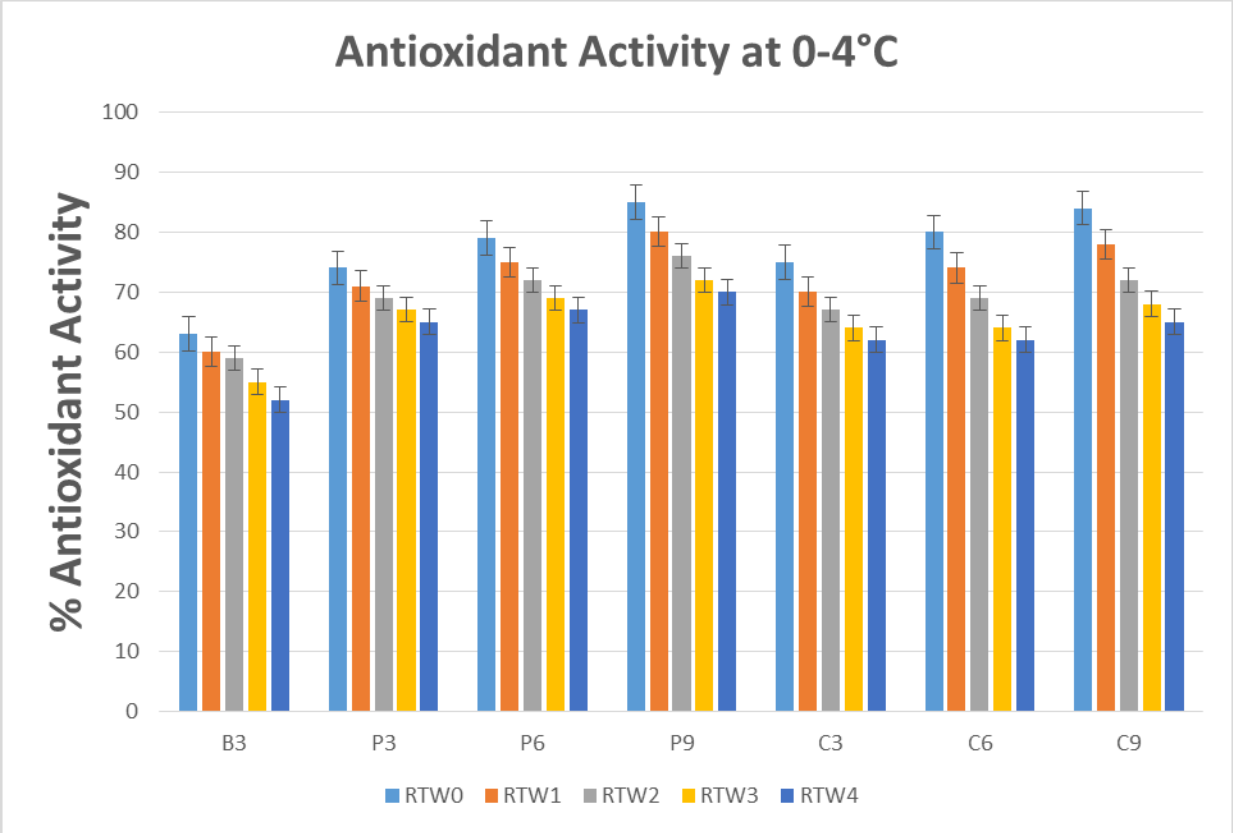


Figure 4.13 Antioxidant activity of radish at 0 – 4 °C

The results indicated that the content of phenolic compounds, in terms of antioxidant activity, of PL and UV-C treated samples increased during storage up to week 1, when compared with the untreated samples. Park and Kim (2015) depicted that UV irradiation, as a postharvest treatment on fresh produce, has been proven beneficial to increase antioxidant activity in different fruits and vegetables. Similarly, the results of pulsed light treated radish displayed significant increase in the antioxidant activity compared with UV-C light treated radish. The result of statistical analysis depicted in Table 4.5.

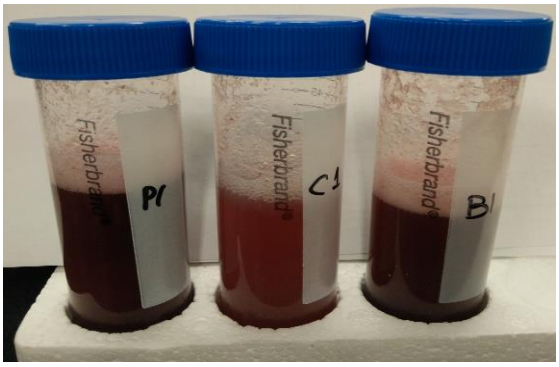
Table 4.5 t-Test: Two-sample assuming equal or unequal variances for radish's antioxidant activity

Storage	Radish types	Variance (F test)	t-stat	t Critical two-tail
Room temperature	x1-y1 (s)	Equal	-5.50	2.31
	x1-z1 (s)	Equal	2.49	2.31
	x1-y2 (s)	Equal	4.64	2.31
	x1-z2 (ns)	Equal	0.99	2.31
	x1-y3 (s)	Equal	4.35	2.31
	x1-z3 (ns)	Unequal	0.58	2.57
10-15 °C	x1-y1 (s)	Unequal	5.00	2.31
	x1-z1 (s)	Equal	2.92	2.31
	x1-y2 (s)	Equal	4.84	2.31
	x1-z2 (ns)	Equal	2.03	2.31
	x1-y3 (ns)	Equal	5.18	2.31
	x1-z3 (ns)	Unequal	1.91	2.57
0-4 °C	x1-y1 (s)	Equal	-4.59	2.31
	x1-z1 (s)	Equal	3.27	2.31
	x1-y2 (s)	Equal	5.07	2.31
	x1-z2 (s)	Equal	3.14	2.31
	x1-y3 (s)	Equal	5.64	2.31
	x1-z3 (s)	Equal	3.96	2.31

This study has shown the positive impact of PL treatment over the UV-C treatment on the quality of the whole radish at an appropriate storage temperature. Therefore, these results supported the PL and UV-C treatments' potential to enhance the accumulation of health-beneficial food compounds in radish without significant changes in the physical properties during storage.

4.3.5 Peroxidase activity

The peroxidase enzyme did not observe to get deactivated post treatment with PL and UV-C at experimented energy level and continued to remain active throughout the shelf life at the experimented storage conditions.



P1: Pulse Treated, C1: UV-C Treated, and B1: Control untreated

Figure 4.14 Peroxidase activity observation

4.3.6 Microbiological study of aerobic and yeast & mold count with storage time

The comparative experiment was carried out to study the effectiveness of PLT versus UV-C treatment as a non-thermal decontamination technology on radish. The trend showed an increase in the aerobic count with the increase in storage time for all storage conditions on both treated and untreated radish. Further, the aerobic count was found higher at room temperature compared with controlled storage temperature. The observations depicted in Figures 4.15, 4.16 and 4.17.

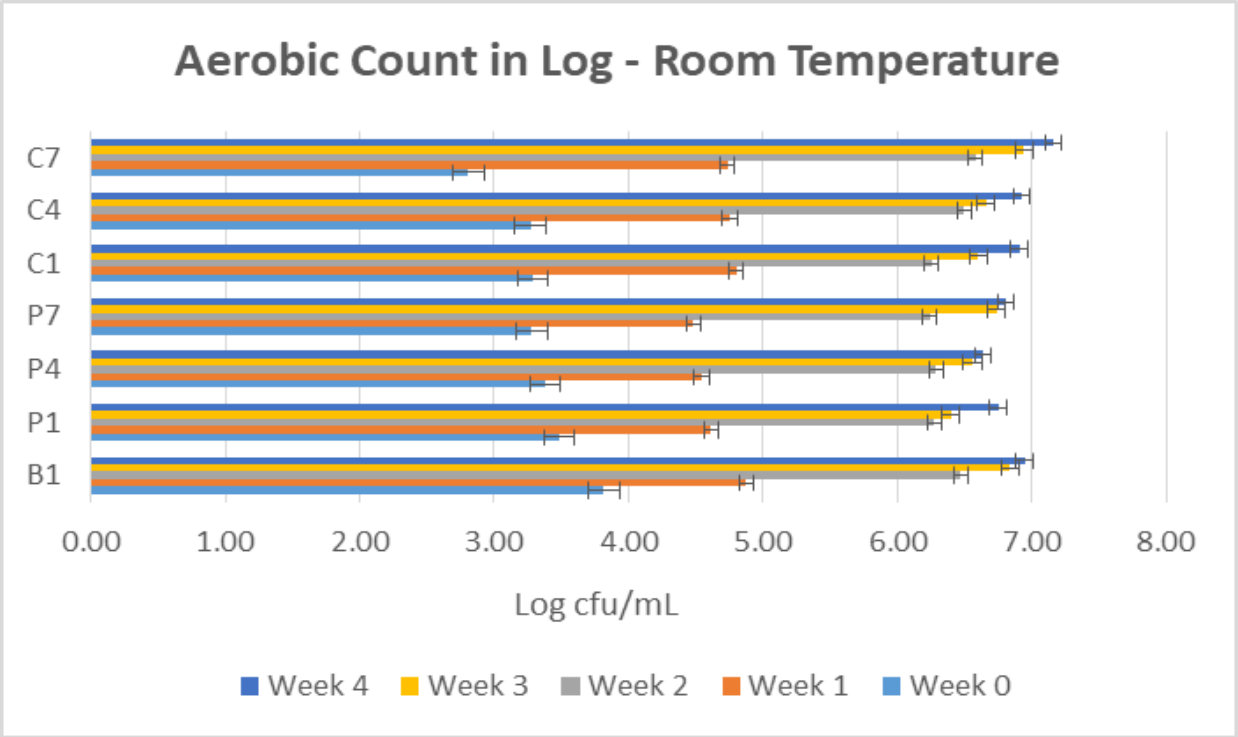


Figure 4.15 Comparison of Aerobic count in log reduction for Radish at room temperature

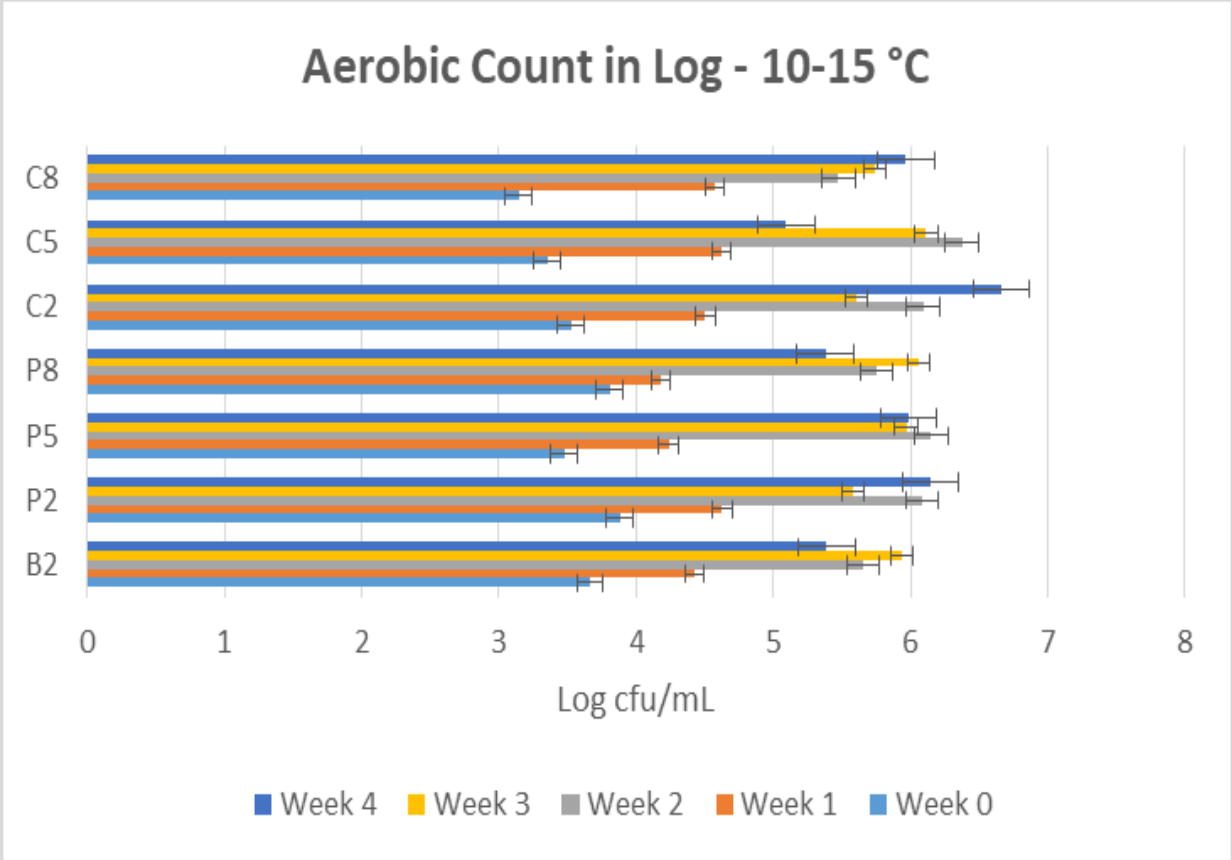


Figure 4.16 Comparison of Aerobic count in log reduction for Radish at 10-15°C

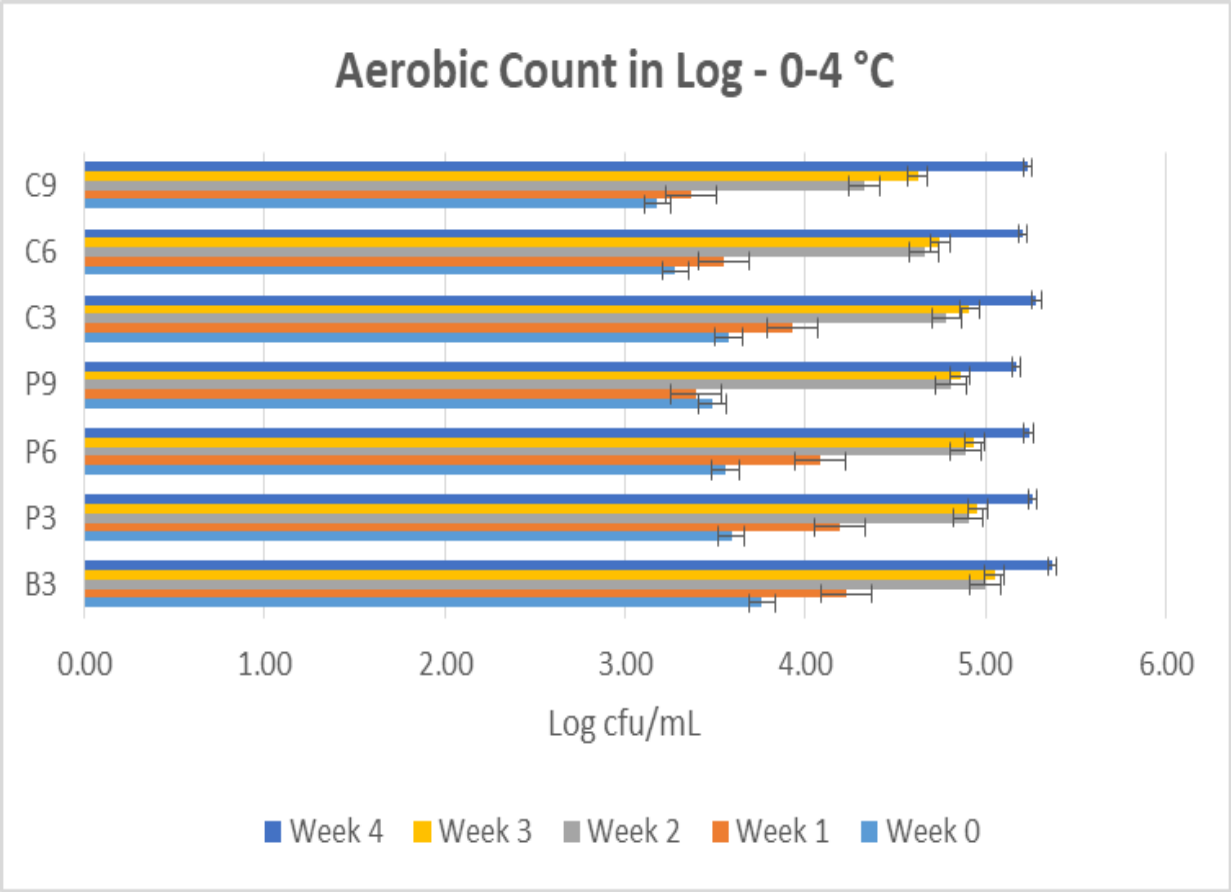


Figure 4.17 Comparison of Aerobic count in log reduction for Radish at 0-4°C

The post-treatment results showed a linear trend in the log reduction of the aerobic count with an increase in energy level, in other words, the higher log reduction was observed followed by the increase in the level of energy (from 2.25 to 6.75 J/cm^2 for UV-C or from 3 pulses/1 sec to 9 pulses/3 sec for PL). At week 0, pulsed light treated radish with 6.75 J/cm^2 energy showed 0.54 log reduction, while a log reduction of 1.01 observed for UV-C treated radish. The results, further, revealed a similar trend until the end of week 2 at room temperature. Therefore, higher log reduction could be achieved by increasing the treatment time. The result of statistical analysis depicted in Table 4.6.

Table 4.6 t-Test: Two-sample assuming equal or unequal variances for radish's aerobic count

Storage	Radish types	Variance (F test)	t-stat	T Critical two-tail
Room temperature	x1-y1 (ns)	Equal	-0.33	2.31
	x1-z1 (ns)	Equal	-0.24	2.31
	x1-y2 (ns)	Equal	-0.34	2.31
	x1-z2 (ns)	Equal	-0.18	2.31
	x1-y3 (ns)	Equal	-0.30	2.31
	x1-z3 (ns)	Equal	-0.14	2.31
10-15 °C	x1-y1 (ns)	Equal	0.10	2.31
	x1-z1 (ns)	Equal	0.43	2.31
	x1-y2 (ns)	Equal	0.43	2.31
	x1-z2 (ns)	Equal	0.49	2.31
	x1-y3 (ns)	Equal	0.58	2.31
	x1-z3 (ns)	Equal	-0.41	2.31
0-4 °C	x1-y1 (ns)	Equal	-0.24	2.31
	x1-z1 (ns)	Equal	-0.42	2.31
	x1-y2 (ns)	Equal	-0.32	2.31
	x1-z2 (ns)	Equal	-0.83	2.31
	x1-y3 (ns)	Equal	-0.71	2.31
	x1-z3 (ns)	Equal	-1.10	2.31

However, at room temperature, the aerobic log reduction decreased with an increase in the energy level, especially the lowest result was observed for UV-C treated radish. This could be explained by shelf life study as the intensity of treatment contributed to the favorable spoilage condition after week 3 which caused the increase in the count of the aerobic count. The following Figures (4.18, 4.19 and 4.20) and Table 4.7 summarizes the findings.

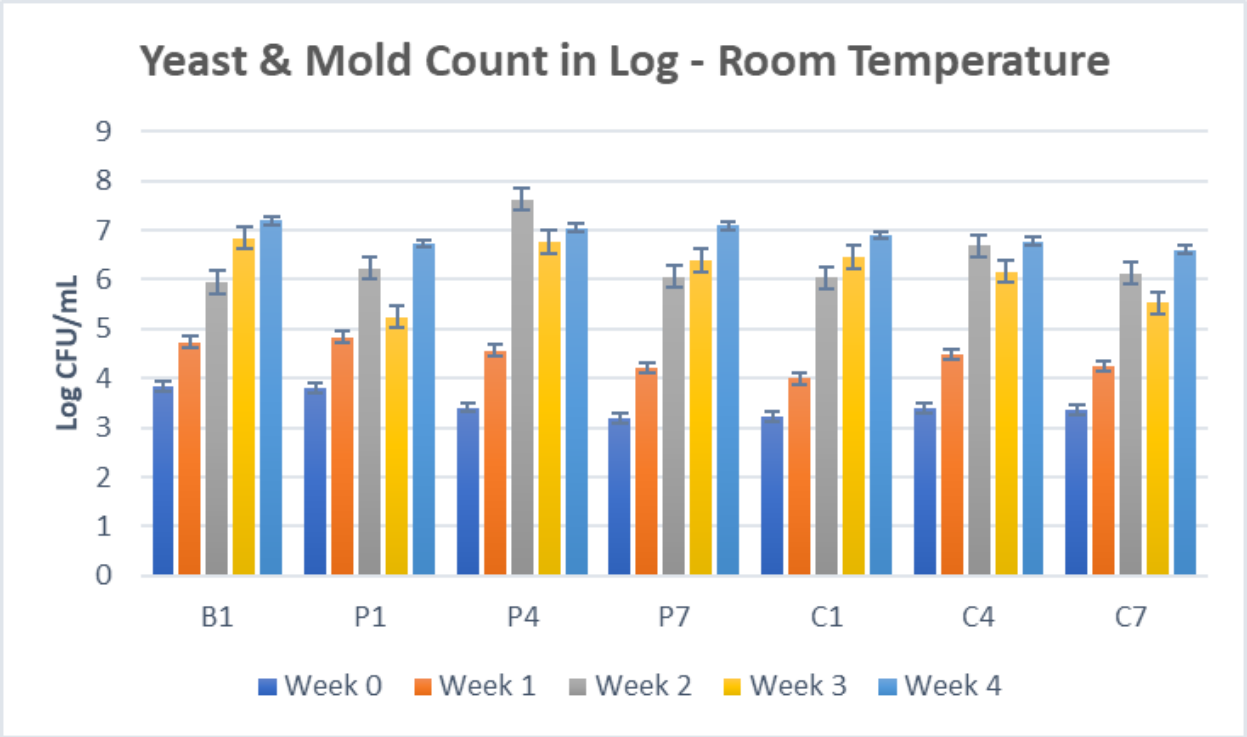


Figure 4.18 Comparison of yeast and mold count in log reduction for radish at room temperature

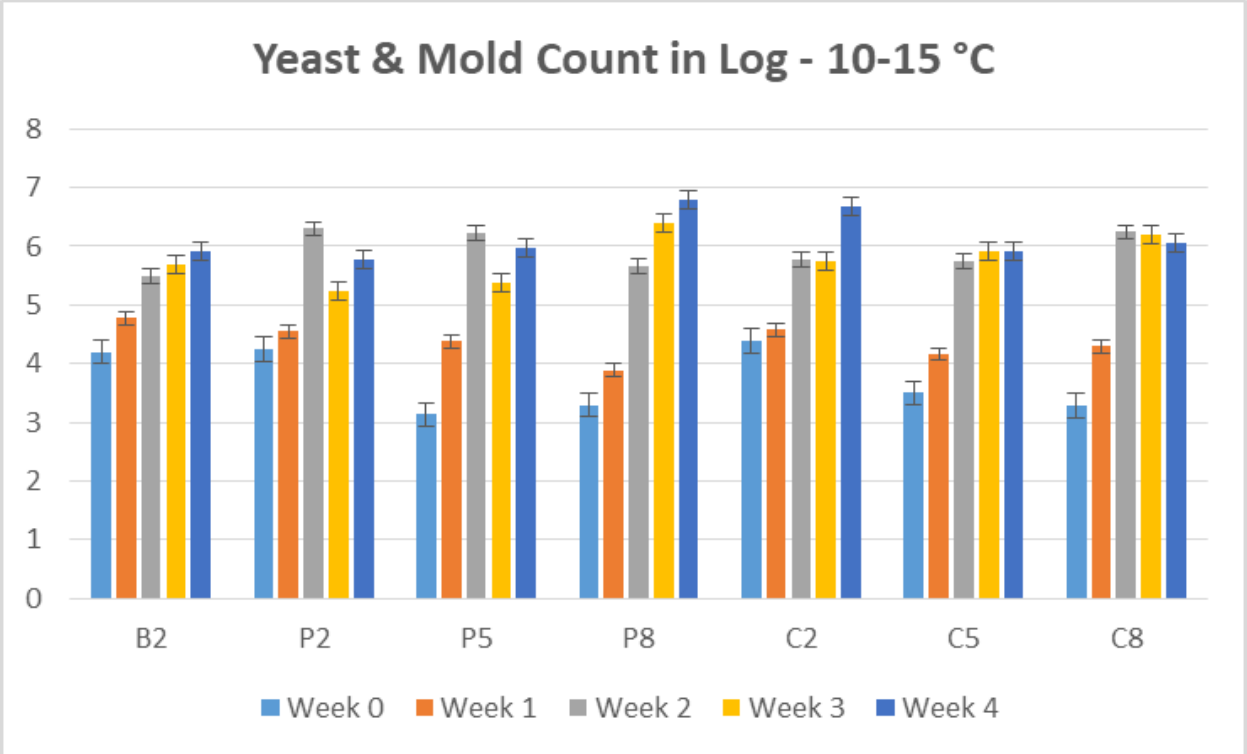


Figure 4.19 Comparison of yeast and mold count in log reduction for radish at 10-15 °C

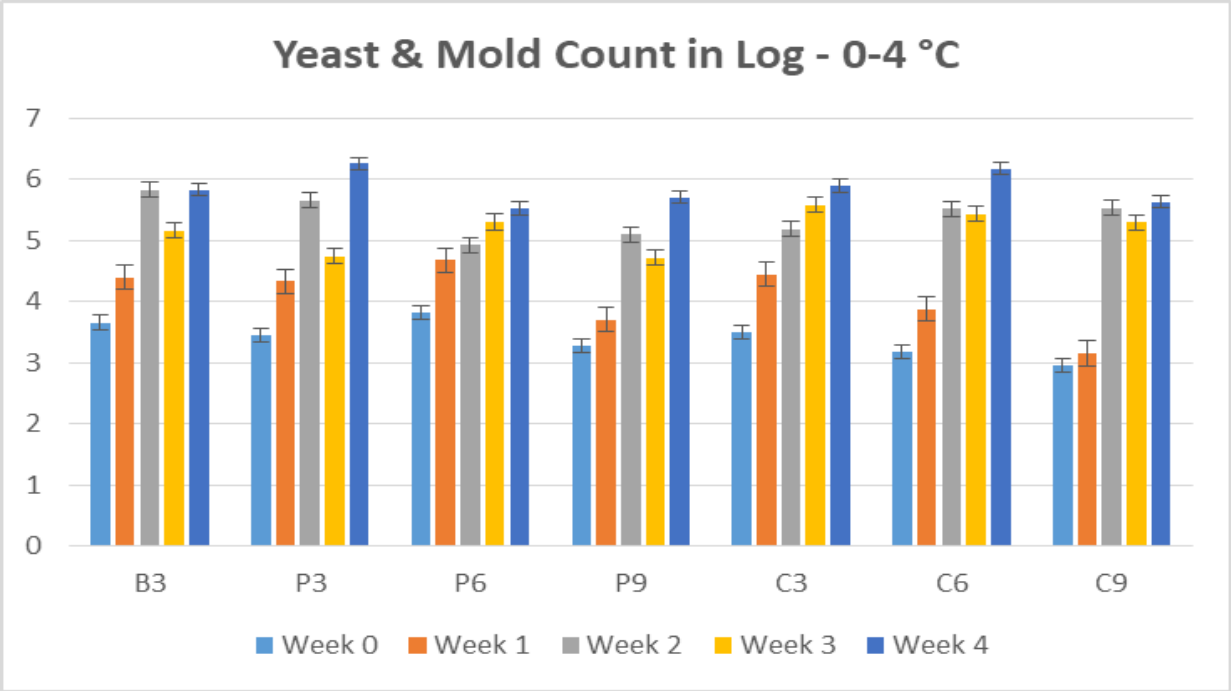


Figure 4.20 Comparison of yeast and mold count in log reduction for radish at 0-4 °C

Table 4.7 t-Test: Two-sample assuming equal or unequal variances for radish's yeast & mold count

Storage	Radish types	Variance (F test)	t-stat	t Critical two-tail
Room temperature	x1-y1 (ns)	Equal	0.42	2.31
	x1-z1 (ns)	Equal	-0.41	2.31
	x1-y2 (ns)	Equal	0.17	2.31
	x1-z2 (ns)	Equal	-0.23	2.31
	x1-y3 (ns)	Equal	-0.33	2.31
	x1-z3 (ns)	Equal	0.61	2.31
10-15 °C	x1-y1 (ns)	Equal	0.02	2.31
	x1-z1 (ns)	Equal	0.42	2.31
	x1-y2 (ns)	Equal	-0.29	2.31
	x1-z2 (ns)	Equal	-0.28	2.31
	x1-y3 (ns)	Equal	-0.01	2.31
	x1-z3 (ns)	Equal	0.01	2.31
0-4 °C	x1-y1 (ns)	Equal	-0.13	2.31
	x1-z1 (ns)	Equal	-0.09	2.31
	x1-y2 (ns)	Equal	0.24	2.31
	x1-z2 (ns)	Equal	-0.20	2.31
	x1-y3 (ns)	Equal	-0.77	2.31
	x1-z3 (ns)	Equal	-0.64	2.31

4.4 Conclusions

The treatments (pulsed light and UV-C) were found positively impacted on the quality in terms of antioxidant activity and microbial log reduction of the radish compared to the untreated radish. Importantly, the pulsed light treated radish showed better results than UV-C treated radish comparatively on the above. This could have been occurred due to the nature of the technology where UV-C effects by means of continuous energy while pulsed light effects by means of intermittent energy. Due to continuous energy, there are significant initial results were observed, but during the shelf-life duration, this form of energy resulted in contributing rapid spoilage in the radish. However, changes in the overall shelf life of the whole radish were minimally affected by the treatments. Furthermore, the rapid rate of physiological spoilage was observed with the increase in treatment energy level which caused damaged to the outer cells.

The study for radish showed that higher log reduction was achieved for yeast and mold count with the increase in energy of UV-C and PL doses. The result of PL treated radish with 2.25 J/cm^2, 4.5 J/cm^2, and 6.75 J/cm^2 energy showed log reduction in yeast and mold counts were 0.46, 0.79, and 0.38 at room temperature, 10-15 °C, and 0-4 °C respectively. Whereas, UV-C treated radish demonstrated the 0.65, 0.91, and 0.71 log reduction with equivalent energy level of PL. The primary reason behind the higher initial log reduction for UV-C treated radish was due to the continuous energy which has contributed to reducing the yeast and mold count. But at a later stage, the initial intensity of this treatment caused the spoilage which showed higher yeast and mold count after week 2.

The PL treatment caused a lower rate of change in pH compared to UV-C treatment during the storage period for the treated radish. But there was not significant difference noticed in a pH change for the treated radish compared to the untreated radish. In addition, treatments increased the moisture loss towards the end of the shelf-life compared to untreated sample. Whereas, for the sample post-treated with the PL and UV-C, the peroxidase enzyme did not get deactivated at the experimented energy level.

Moreover, the result of the PL treated radish demonstrated enhance in the percentage of antioxidant activity by increasing their total polyphenolic content, whereas UV-C treated radish showed a lesser increase in the percentage of antioxidant activity compared to PL treated radish. This illustrates the potential use of pulsed light to enhance the nutritional content of radish. The

antioxidant activity of radish stored at 0-4 °C on week 0 showed an increase of 85% after treated with PL at 6.75 J/cm^2, while it was 84% in case of UV-C treated radish equivalent to 6.75 J/cm^2. Similarly, for stored at room temperature and 10-15 °C, the results found 80% and 82% after PL treatment, and 79% and 78% after UV-C treatment respectively.

Thus, a pulsed light treatment found better compared to UV-C treatment and so it can be considered as a surface microflora decontamination technology to achieve effective inactivation of micro-organisms. In conclusion, this comparative study has shown its usefulness in identifying desirable treatment conditions to achieve targeted quality in radish.

CHAPTER 5
GENERAL CONCLUSIONS AND FUTURE RECOMMENDATIONS

GENERAL CONCLUSIONS

1. Minimal changes in the overall appearance during the shelf life of PL treated whole carrot were observed compared to PL treated cut carrots with total microflora Log reduction from 0.12-0.42 log.
2. The antioxidant activity of whole carrots showed an increase of 85% after treatment with PL at 11.25 J/cm^2 at the storage environment. Whereas, the cut carrots after treatment to PL treatments of 1 s and 3 s showed 89% and 91% increase in antioxidant activity.
3. In the case of radish, the overall appearance of treated radish was found to change during shelf life studied with total microflora Log reduction from 0.12-1.07 log.
4. The study for radish showed that higher log reduction was achieved for yeast and mold count with the increase in energy of UV-C and PL doses. The result of PL treated radish with 2.25 J/cm^2, 4.5 J/cm^2, and 6.75 J/cm^2 energy showed log reduction in yeast and mold counts were 0.46, 0.79, and 0.38 at room temperature, 10-15 °C, and 0-4 °C respectively. Whereas, UV-C treated radish demonstrated the 0.65, 0.91, and 0.71 log reduction with equivalent energy level of PL.
5. The antioxidant activity of radish stored at 0-4 °C on week 0 showed an increase of 85% after treated with PL at 6.75 J/cm^2, while it was 84% in case of UV-C treated radish equivalent to 6.75 J/cm^2. Similarly, for stored at room temperature and 10-15 °C, the results found 80% and 82% after PL treatment, and 79% and 78% after UV-C treatment respectively.

RECOMMENDATIONS FOR FUTURE RESEARCH

This research unfolded several key findings. Further, it showed some ideas of interest for future research and development, which could be summarized as follows:

1. This research can be further extended to understand the adverse effects on specific commodities as UV light can generate free radicals in certain food products.
2. The design and commercial application of this technology needs to be carefully determined to avoid any exposure of the germicidal wavelength on to the processing employees.

3. Consumer acceptability towards any irradiation technology is not very well appreciated in the current time. Even if this technology has become successful in achieving the required objective, a lot more needs to be done from regulatory, health, and safety standpoint to increase awareness and acceptability among consumers. Research data on this topic will help in technological acceptability amongst the consumer.

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