The Use of Light Emitting Diodes (LEDs) for Shelf Life Extension of Spinach and Kale

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Abstract

The spectral composition of light-emitting diodes (LEDs) has been reported to result in higher yield, prevent wilting, and reduce thermal damage to plants. The use of LEDs for postharvest storage and shelf life extension has been limited, but the potential of this technology will allow for greater applications in horticulture and the food industry. In this experiment, 'Winterbor' kale (*Brassica oleracea* L.) and 'Melody' spinach (*Spinacia oleracea* L.) plants were measured for photosynthesis response and stomata response under 14 different wavelengths of light. The data collected from the measurements were used to select two different wavelengths of LEDs and determine the proper irradiance levels for an LED irradiance storage test on spinach and kale. The hypothesis was that by selecting a wavelength that results in stomata closure and selecting an irradiance level at the light compensation of the plant, the plants can be stored successfully for a longer period, compared to conditions under fluorescent light or no light. Treatments of the blue lights (ranging from 405 nm to 470 nm), red lights (ranging from 624 nm to 661 nm), and amber light (595 nm) were effective at increasing the stomatal opening, while the green lights (ranging from 501 nm to 560 nm) resulted in reduced stomatal opening. For spinach, the light response curve resulted in 500 nm and 560 nm having a light compensation point at 65.3 and 64.7 μmol m^{-2} s^{-1}, respectively. For kale, the light compensation points of 500 nm and 560 nm were at 50.8 and 44.1 μmol m^{-2} s^{-1}, respectively. For the storage test experiment at room temperature, kale and spinach were stored under four different treatments: dark treatment as a control, standard white fluorescent light (at 50 μmol m^{-2} s^{-1}), and 500 nm and 560 nm LED wavelengths (at 55 μmol m^{-2} s^{-1}). Shelf life testing of kale resulted in the lowest moisture loss of 40% at 560 nm treatment and 41% moisture loss for spinach. The control (dark) had the highest moisture loss at 54% for kale and 52% for spinach. A visual assessment scale was monitored throughout the experiment and results showed a better visual quality in kale under 560 nm compared to the lowest visual quality under the dark treatment by day 4. For spinach, the visual quality for 560 nm treatment followed a similar pattern as fluorescent and 500 nm, resulting in a poor-quality product by day 4 and the lowest-quality product by day 5. The LED treatments improved the shelf life of spinach and kale which was the resultant of stomatal aperture closure, photosynthetic rate near light compensation point and stability of atmospheric moisture content.

Résumé

La composition spectrale de diodes électroluminescentes (LED) a été rapportée pour offrir un rendement supérieur, prévenir la flétrissure et réduire les dommages thermiques aux plantes. L'effet de l'utilisation de LED pour l'entreposage après récolte sur la prolongation de la durée de conservation a été limité, mais le potentiel de cette technologie permettra une plus grande application dans l'horticulture et l'industrie alimentaire. Dans cette expérience, le chou frisé 'Winterbor' (*Brassica oleracea* L.) et les épinards 'Melody' (*Spinacia oleracea* L.) ont été mesurés pour la photosynthèse et réponse sous 14 différentes longueurs d'onde de lumière. Les données recueillies à partir des mesures ont été utilisées pour sélectionner deux différentes longueurs d'onde de LED et déterminer le niveau d'irradiance LED pour un test d'entreposage avec des épinards et du chou frisé. L'hypothèse était qu'en sélectionnant une onde qui entraîne la fermeture des stomates et la sélection d'un niveau d'éclairement à la compensation de la lumière de la plante, les plantes peuvent être entreposées avec succès pour une période plus longue, par rapport aux conditions en lumière fluorescente ou sans lumière. Les traitements à la lumière bleue (allant de 405 nm à 470 nm), la lumière rouge (allant de 624 nm à 661 nm), et jaune (595 nm) sont efficaces pour augmenter l'ouverture des stomates, tandis que la lumière verte (allant de 501 nm à 560 nm) a permis de réduire l'ouverture des stomates. Pour les épinards, la courbe de réponse de lumière a s'est située à 500 nm et 560 nm, possédant un point de compensation de lumière à 65.3 et 64.7 $\mu mol\ m^{-2}\ s^{-1}$, respectivement. Pour les choux, les points de compensation de lumière de 500 nm et 560 nm ont été de 50.8 et 44.1 $\mu mol\ m^{-2}\ s^{-1}$, respectivement. Pour l'entreposage à température ambiante, le chou frisé et les épinards ont reçu quatre différents traitements : traitement sombre comme contrôle, éclairage fluorescent blanc standard (à 50 $\mu mol\ m^{-2}\ s^{-1}$), et 500 nm et 560 nm (à 55 $\mu mol\ m^{-2}\ s^{-1}$). La durée de conservation du chou a eu le taux de perte d'humidité de 40 % à 560 nm et de 41 % de perte d'humidité pour les épinards. À la noirceur la perte d'humidité est la plus élevée à 54 % pour le chou frisé et 52 % pour les épinards. Une évaluation visuelle a été effectuée tout au long de l'expérience et les résultats ont montré une meilleure qualité visuelle du chou sous 560 nm par rapport à la plus faible qualité visuelle sous l'obscurité après 4 jours. Pour les épinards, la qualité visuelle à 560 nm suit la même tendance que les lampes fluorescentes et à 500 nm, ce qui entraîne une mauvaise qualité de produit après 4 jours avec la plus faible qualité de produit au jour 5. Les traitements LED ont eu une incidence sur la durée de conservation et la qualité post-

récolte des épinards et du chou frisé qui a été la résultante de l'ouverture des stomates, réponse photosynthétique, et la mesure des paramètres de stabilité d'humidité atmosphérique.

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Abbreviations

ABA	Abscisic acid
ATP	Adenosine trisphophate
CAM	Crassulacean acid metabolism
Ca^{2+}	Calcium ions
CO_2	Carbon dioxide
HID	High intensity discharge
HPS	High pressure sodium
K^+	Potassium ions
LED	Light emitting diode
$NADP^+$	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide hydrogen
mmol	Milli moles
nm	Nanometers
O_2	Oxygen gas
PAR	Photosynthetically active radiation
W	Watts
μmol	Micro moles

1. General Introduction

1.1. Thesis Motivation

More than $7 billion of fresh produce is wasted in Canadian grocery stores every year (Vuchnich, 2015). According to Agriculture and Agri-Food Canada, Canadian homes and the retail industry have lost and wasted 6 billion kg of food in 2010. In 2014, the yearly cost of Canada's food waste was estimated to be $31 billion and it was estimated that $21 billion worth of food was composted and landfilled (Gooch, Felfel and Marenick, 2010). The produce thrown out had lost its appeal and marketability due to changes in color, loss of turgor pressure (limpness), cell death (necrosis) and mold. The use of light-emitting diodes (LEDs) for shelf life extension can be used to limit these effects by reducing food spoilage and enhancing nutritional quality. LEDs are appropriate for postharvest storage due to their low radiation heat emission and increased efficiency at low temperatures. The Canadian agricultural sector and agri-food system, which accounted for nearly $87.9 billion of the economy in 2006 (Kittson et al., 2008), is faced with challenges in food security and postharvest safety. The sector is working to decrease food loss and waste produced by incorporating waste management techniques that provide good quality produce for consumption and protect the environment from excess waste. This research will provide a solution to extend the shelf life of leafy greens all year round. Reducing fresh produce waste in grocery stores will increase revenue, thereby benefiting the Canadian economy while also providing social and environmental benefits that entail increased food security and reduced food waste.

1.2. Research Problem

Most LED studies have focused on using LEDs to optimize plant growth in controlled environments. However, the use of LEDs to improve the appearance of consumable food has not been studied extensively. Existing studies have focused on using other parameters such as fungal species and ethylene production as factors impacting plant storage (D'Souza et al. 2015). LEDs have been tested for plant growth and have been reported to result in higher yields, the reduction

of wilting, and the reduction of thermal damage to plants. However, the use of LEDs for postharvest storage and shelf life extension has been limited.

1.3. Objectives

- Identify a LED wavelength appropriate for long-term storage experiments.
- Analyze stomata response to different LED wavelengths and use the data to test the wavelengths' ability to extend shelf life.
- Measure photosynthetic rates of plant species to determine the light compensation point needed for shelf life testing.
- Estimate an appropriate wavelength for kale and spinach to be used for further experimentation.
- Set specific LED wavelength treatments, intensities and photoperiods to provide successful postharvest storage while integrating appropriate environmental conditions.

1.4. Hypothesis

- Selecting a wavelength that results in decreased stomatal aperture and photosynthetic rate will successfully extend the shelf life of spinach and kale.
- The green LED wavelengths will result in decreased stomatal aperture size and lower photosynthetic rate.
- Determining the light compensation point of the chosen wavelength will improve postharvest quality and will result in better visual quality compared to leafy greens stored in the dark (control).

2. Literature Review

2.1. Photosynthesis

The sun provides the energy needed by all life on earth. Photosynthesis is the chemical process that converts this energy to chemical energy. Photosynthesis (Eq.1) is a reduction-oxidation chemical reaction in which light energy is used by plants and photoautotroph organisms (such as bacteria, and algae) to oxidize water molecules, form sugars and release oxygen (Taiz and Zeiger, 2002):

$$6\ CO_2 + 6\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2 \qquad (1)$$

The plant's photosynthetic process occurs in the mesophyll which contains the chloroplast. The stroma surround the grana in the chloroplast and within the stroma are sacks called thylakoids, where photosynthesis occurs. Within the thylakoid membranes are five protein complexes responsible for electron transport and synthesis of energy carrier molecules NADPH and ATP (Cooper, 2000).

2.1.1. Photochemical reaction centers and light harvesting antennae complexes

Photosynthesis requires two protein complexes to carry out the primary light reactions, photosystem I (PS I) and photosystem II (PS II). These photosystems possess a photochemical reaction center and a light harvesting antenna complex responsible for the absorption of light and the transfer of energy to the reaction center. The main pigment in photosynthesis is chlorophyll a (Chl *a*) and is present in the light reaction center in all living organisms (Farabee, 2007). The two photosystems are linked through an electron transport system, they are similar in functions but differ in absorption profiles: PS I reduces $NADP^+$ and absorbs in the far-red light (wavelengths <680 nm) whereas PS II oxidizes water and absorbs at 680 nm (Taiz and Zeiger, 2002). In the PS II reaction center, Chl *a* is known as P-680 due to its peak absorption wavelength and Chl *a* is known as P-700 in PS I. (Mishra, 2004). Chlorophyll b (Chl *b*), carotenoids, β-carotene and

carotenoid subsets (such as xanthophyll, lutein, violaxanthin, anteroxanthin and zeaxanthin) are accessory antennae pigments that are highly preserved in higher plants. The carotenoids are secondary pigments that are synthesized in plants, fungi and bacteria (Sandmann, 2001) and harvest light energy and channel it through resonance energy transfer to the reaction center (Lockstein, 2007).

The photosynthetic light reaction begins when light energy excites electrons in Chl *a* pigment in PS II, oxidizing electrons. The electrons are split into 2 hydrogen ions (H^+) and free oxygen (O^{2-}) ions. The O^{2-} ions form diatomic O_2 and the protons in the thylakoid lumen form a proton gradient across the thylakoid membrane, ultimately resulting in ATP synthesis (Farabee, 2007). After energy is absorbed and transferred to the reaction center of PS II, the electron transport chain in photosystem transports energized electrons to the plastiquinone (PQ). As PQ transports the electrons to the cytochrome *bf6*, a second protein complex, the electrons lose energy and pump protons in the thylakoid lumen. Plastocyanin transfers the electrons to the reaction center of PS I (Cooper, 2000). Ferrodoxin transports the electrons to NADP reductase, where $NADP^+$ is reduced to NADPH. ATP synthase converts ADP and inorganic phosphate to ATP (Taiz and Zeiger, 2002). In the stroma of the chloroplast, NADPH and ATP fuel carbon fixation in the Calvin cycle reactions mediated by Rubulose-1,5-biphosphate carboxylase (RuBisCo) (Taiz and Zeiger, 2002).

2.1.2. Photosynthesis Action Spectrum

Wavelengths used by plants for photosynthesis are within the visible spectrum (ranging from 400 to 700 nm); the spectrum is known as photosynthetically active radiation (PAR) (McCree et al., 1972). The photosynthesis action spectrum is defined as the rate of photosynthetic efficiency as calculated in Equation 2. Different wavelengths of light result in different photosynthetic efficiencies (McCree et al., 1972).

$$\text{Action spectrum} = (P_{(light)} - P_{(dark)})/\text{irradiance} \quad (2)$$

$P_{(light)}$ is light net photosynthesis in mmol CO_2 m^{-2} s^{-1}, $P_{(dark)}$ is net photosynthesis in darkness in mmol CO_2 m^{-2} s^{-1} and irradiance in μmol m^{-2} s^{-1}.

Quantum yield (Eq. 3) is the photosynthetic utilization efficiency expressed in photon absorption by leaf pigments (McCree et al., 1972).

$$\text{Quantum yield} = \text{action spectrum} / (\text{wavelength} \times \text{absorption}) \quad (3)$$

Where, wavelength is the irradiance of a given wavelength and absorption is the wavelength absorbed, expressed in %.

Action spectrum peaks occur at 450 and 675 nm in a single action spectrum obtained from 22 plant species (McCree et al., 1972). 450 and 675 nm correspond to the absorption of Chl *a* and *b*. A peak at 437 nm and a maximum at 670 nm were reported in kidney beans irradiated with different wavelengths of light (Balegh et al., 1970). Hoover et al. (1937) irradiated wheat and measured a peak at 435 nm and a maximum at 677 nm. Bulley et al. (1969) conducted a similar study on corn and radish leaves and showed a peak at 440 nm and a maximum at 660 nm for both plant species.

Different factors impact the action spectrum such as plant species and irradiance, amongst many others (McCree et al., 1972). The difference between absorption spectra of leaves is what causes different effects of plant species on action spectrum (McCree et al. 1972). The concentration and absorption of leaf pigments and leaf internal and external structure impact the action spectrum. Irradiance determines the action spectrum due to the correlation that irradiance and photosynthetic activity is dependent on wavelength (McCree et al., 1972). Red and blue light have been determined to be the optimum wavelengths to drive photosynthesis (Folta and Marunhnich, 2007). These light qualities are preferred due to their efficiency in developing autotrophic growth characteristics (Folta and Marunhnich, 2007).

Green light (500 – 600 nm) has distinct effects on plant growth. It has been shown to have an effect on plant processes via the cryptochrome (Folta and Maruhnich, 2007). These receptors are sensitive to all light qualities and establish responses to low quantities of light from wavelengths across the spectrum. Phytochromes and cryptochromes are green light receptors (Folta and Maruhnich, 2007) but their ability at processing green light is not as efficient as their response to blue and red light. In general, the effects of blue and red light are contrary to those of green light. The cryptochromes control blue and UV-A light responses (Lin, 2002), and recent evidence show biological activity of green light-sensing state (Bouly et al., 2007). Cryptochromes

depend on intramolecular electron transfer for their signalling mechanism (Malhotra et al, 1995). The green absorbing state of cryptochrome 1 (cry1) and cryptochrome 2 (cry2) reverses responses influenced by blue light (Bouly et al., 2007). There is at least another light sensor system that drives specific effects to green light. These responses can be characterized as cryptochrome dependent and cryptochrome independent (Folta and Maruhnich, 2007). Bouly et al. (2007) showed that 564 nm green light has the ability to inhibit the blue light cryptochrome activation and reverse the blue light hypocotyl growth inhibition in developing seedlings of Arabidopsis. Bouly et al. (2007) stated that this effect is cry-dependent, in accordance with green-blue reversibility model. Responses to pulses of blue light through cry2 accumulation were tested by irradiating blue light pulses followed by finite green light pulses. The results show that short pulses of green light can reverse the blue light degradation of cry2.

Steinitz et al. (1985) stated that blue light receptor mediating phototropism is responsible for the green light response, phototropin 1 (phot1). Green light was more active in wavelengths closer to the blue spectrum; 510 nm was more effective than 520 nm and 550 nm. The latter wavelengths required significant irradiation to produce the same degree response of shorter wavebands of green light (Steinitz et al., 1985).

2.1.3. Light emitting diodes (LEDs) as electrical lighting systems

The use of electrical lighting for cultivation in controlled environments allows for steady plant productivity independent of external weather patterns. LEDs are now being used largely as lighting systems for horticultural facilities (D'Souza et al., 2015). LEDs are an effective lighting source for crop production due to characteristics such as small size, compactness, durability and the low cost (Mitchell and Stutte, 2015). In addition, LEDs have narrow wavebands and cooler emitting surfaces. The spectral composition, intensity and temporal settings of LEDs are easily controlled (Branas et al., 2013). They have a range of luminous efficacy that resemble fluorescent and high intensity discharge (HID) lights and their efficiency is continuously increasing (U.S. Dept. of Energy, 2013). Nelson and Bugbee (2014) reported that LEDs have a photon efficiency and electrical efficiency similar to high pressure sodium (HPS) lights and slightly higher than fluorescent lights. LEDs have a life expectancy between 50,000 to 100,000 hours, whereas conventional lighting technologies have a life expectancy of 15,000 hours (Gupta and Jatothu,

2013). In addition, unlike most conventional lighting systems, LEDs have the ability to pulsate or be dimmed to better control the emission of light on plants according to specific physiological responses (Yeh and Chung, 2009). Fluorescent, incandescent, and HID lighting have a broad-spectrum distribution, and due to the presence of heavy metals in fluorescent lamps, the lights need to be handled carefully (Lui et al., 2014). Conventional lighting systems generate more heat and require more complex heat management systems in controlled environments whereas LEDs have more focused heat loads that are more easily managed (Mitchell et al., 2012). Recent studies of small scale horticulture have shown an improvement in plant productivity and nutritional content with the use of LEDs (Morrow, 2008; Lefsrud, 2006). LEDs emit less radiation as heat, which minimizes the thermal radiation on crops generated by traditional light sources (Morrow, 2008; Mitchell et al., 2012), and reduces the allowable distance between the plant and the light source, thereby increasing light efficiency (D'Souza et al., 2015).

2.1.4. Photosynthetic response to LED light quality

Plants respond to light through numerous pathways, depending on wavelength and irradiance level. The phytochrome system reacts to light in the 350 – 800 nm spectrum and adjusts metabolic and photomorphological responses (Dorais, 2003). Current literature on photomorphogenesis and photosynthesis mainly focuses on the effects of blue and red light, as green light has been considered insignificant. In addition to blue and red, other wavelengths play an important role in plant growth but sometimes respond in opposing ways to each other, such as green light counteracting the effect of blue light (Frechilla et al., 2000). Research is still required to provide spectral balance to maximize yield of plants (Mitchell and Stutte, 2015).

Blue light (400 – 500 nm) comprises of approximately 33% of sunlight PAR spectrum (Mitchell and Stutte, 2015). A small percentage of blue light is necessary to trigger specific plant responses such as phototropism, stomatal aperture, leaf thickness and chlorophyll content (Briggs and Christie, 2002; Mitchell and Stutte, 2015). Stem elongation and leaf expansion are triggered by blue light but when large amounts of blue light are administered, inhibition of growth can occur (Mitchell and Stutte, 2015). McNellis and Deng (1995) showed that blue light inhibits hypocotyl elongation and promotes the production of biomass.

Green light is usually overlooked when considering wavebands necessary for photosynthesis. Absorption spectra of leaf chlorophyll show weaker absorption in the green spectrum of PAR compared to blue and red spectrum (Mitchell and Stutte, 2015). Intact leaves absorb over 75% of green light (Terashima et al., 2009). Measurement of the relative quantum efficiency for photosynthesis vs PAR wavelengths, specific green wavelengths result in higher photosynthesis than some blue wavelengths but overall broadband blue light is more efficient than broadband green light (Mitchell and Stutte, 2015). Green light influences leaf growth, stomatal conductance and early stem elongation (Folta, 2004; Kim et al., 2004a, b) as it reaches the lower canopy better than the red light and blue light (Klein, 1992). According to Folta (2004), green light promotes stem elongation, decreases biomass production, and promotes plant growth when used as a supplement light to red and blue light (Folta and Maruhnich, 2007; Kim et al., 2004a, b). Terashima et al. (2009) showed that chlorophylls and plant pigments absorbs green light poorly, but that green light increases photosynthesis in sunflower leaves when mixed with white light.

Red light (600 – 700 nm) has the highest quantum efficiency for driving photosynthesis and has a peak from 620 to 660 nm (McCree, 1972). Red light has morphological effects on plant growth driven by the photoreversible pigment, phytochrome (Mitchell and Stutte, 2015). Red light stimulates stem elongation, increased biomass, leaf expansion, flowering, seed germination, dormancy and controls other photomorphogenic responses (Mitchell and Stutte, 2015). The electrical efficiency of red LEDs is higher than fluorescent light (Yorio et al. 2001) yet, in plant growth at the same light intensity, lettuce (Bula et al 1991), spinach, and radish grown under red light alone were not as healthy as plants grown under fluorescent light. Red light regulates hypocotyl elongation and increases leaf area (Johkan et al., 2010).

White light LEDs are phosphor coated blue LEDs. White light is less electrically efficient than monochromatic blue LEDs due to the energy losses from the secondary broadband photon emissions of the excited phosphor (Bourget, 2008). The red, blue and green distribution of white LED light is not a close match to the RGB (red, green, blue) distribution of solar light at midday (Mitchell and Stutte, 2015). White LEDs may be useful to attain some broadband proportions in case green LEDs are not included.

Far-red light (700 – 800 nm) controls functions linked to photoperiodism and photomorphogenesis that involve the phytochrome pigment system (Mitchell and Stutte, 2015). Plant species requiring flowering during long days are treated with far-red simultaneously with red

light (Deitzer et al., 1979). Far-red light impacts plant stem elongation (Li and Kubota, 2009). Plants exposed to far-red radiation develop larger but thinner leaves which allows the far-red light to pass through the upper layer of the leaf canopy (Mitchell and Stutte, 2015).

Blue and Red light combination: To improve plant growth, a combination of blue and red LED light has been explored. Ohashi-Kaneko (2007) conducted an experiment growing rice plants under a combination of red and blue light (80% and 20% respectively). The results showed a higher photosynthetic rate per unit leaf area in young rice leaves treated with blue light compared to plants irradiated with red light (Matsuda et al., 2004). Ohashi-Kaneko (2007) reported that supplementing red light with blue light increases nitrogen levels along with Rubisco, cytochrome *f*, chlorophylls and chl *a/b* ratio.

Both red and blue light are sufficient for plant growth, however it is beneficial to include other wavelengths to improve overall plant development (D'Souza et al., 2015). Given the recent literature, the flexibility of the spectral composition and output of LEDs can provide a better understanding of plant responses to varying light quality.

2.2. Stomata

Stomata facilitate gas exchange, photosynthesis, and minimize water loss during transpiration (Assmann, 1993; Zeiger, 1983; Shimazaki et al., 2007). Stomata respond to changes in the environment, work to conserve biological integrity and are a passage between the plant and the environment (Zeiger, 1983). Guard cells control the stomata opening size by regulating the amount of water vapor loss and gas exchange (carbon dioxide and oxygen). Stomata respond to a variety of internal and external stimuli. Stomatal function is driven by homeostasis (Zeiger, 1983). Stomata close under water stress to limit moisture loss (Ludlow, 1980). Stomata are used by the plant as part of the optimization theory to increase the rate of carbon gain whilst decreasing the amount of water loss (Cowan, 1982; Cowan and Farquhar, 1977; Farquhar and Sharkey, 1982).

2.2.1. Stomatal Movement

Stomata opening and closing are the result of turgor changes in the guard cells (Hsiao, 1976; Meidner and Mansfield, 1968; Cowan and Farquhar, 1977). An increase in turgor pressure increases stomatal aperture due to the mechanism of the cell walls, and, a decrease in turgor pressure decreases stomatal aperture. There are two processes linked to stomatal movements: 1) the alteration of ionic and organic compounds that are dependent on physiological and metabolic conditions, and, 2) water fluxes, turgor pressure changes and guard cells mechanical responses which result in changes in aperture dimensions (Raschke, 1979). Saftner and Raschke (1981) concluded that there are membrane potential differences in guard cells and high concentrations of electrolytes in open stomata.

Stomata Opening: Most plants open in the presence of light and close in the absence of light except for crassulacean acid metabolism (CAM) plants. CAM plants open in the dark and close in the light due to their ability to fix carbon dioxide in the absence of light.

The opening of the stomata causes a decline in water potential and water uptake. An increase of turgor increases the size of the guard cell thereby increasing the aperture size of the stomata. Small vacuoles in closed stomata merge to form larger vacuoles during the opening of the stomata. The changes in turgor pressure of the guard cells are caused by the transport of K^+

into and out of the cells (Fischer, 1968) and an increased K^+ level is present in open stomata compared to closed stomata (Hsiao, 1976). The K^+ concentration in the guard cells is a few folds higher than the concentration around the tissue, affirming that there are concentration mechanisms working against the diffusion gradients (Hsiao, 1976). Humble and Hsiao (1969) demonstrated that there is a selective K^+ uptake among other ions and Ca^{2+} enhanced their selectivity (Hastings and Gutnecht, 1978). K^+ concentrations vary with light, abscisic acid (ABA) and carbon dioxide (CO_2) concentrations (Jarvis and Mansfield, 1980). The K^+ content and the stomatal aperture size are highly correlated (MacRobbie, 1977) and result in half of the osmotic potentials used for the accumulation of turgor pressure in the guard cells (Zeiger, 1983).

Stomata Closure: Under drought and high salinity, ABA levels increase and trigger changes in ion fluxes causing stomatal closure (Araujo et al., 2011). The decrease in K^+ levels in the tissues can be caused by increased levels of abscisic acid (ABA) surrounding the guard cells (Mansfield and Jones, 1971). There is a relationship between ABA levels and stomatal reaction to water stress (Mansfield and Davies, 1981b). Measurements of ABA concentration in the epidermis show that ABA increases in epidermal peels faster than in the mesophyll which proposes that the effects of ABA may depend directly on guard cell metabolism (Weller et al., 1982). Stomatal closure can also be triggered by osmotica being removed from guard cells when the environment is unfavorable i.e. drought stress, elevated CO_2 concentration, low humidity. Epidermal cells are crucial in the redistribution of osmoticum driven by ABA which indicates the effects of ABA on membrane permeability (Itai and Meidner, 1978).

Stomata close when Cl^- is included in the incubation medium (Schnabl, 1981). It is believed that Cl^- acts as a counter ion of K^+ (Penny et al., 1976; Van Kirk and Raschke, 1978) for species that lack starch in their guard cells. Some species with starch present in their guard cells use organic acids such as $malate^{2-}$ (Zeiger, 1983).

2.2.2. Factors Affecting Stomatal Aperture

Stomatal aperture is impacted by various external environmental factors, namely light, CO_2, humidity, temperature (Schulze and Hall, 1982), and internal factors such as water present in the tissues, ABA and cytokinins.

Temperature, Vapor Pressure Deficit and Humidity: The effects of temperature and humidity on stomatal movement are difficult to distinguish because temperature increases with vapor pressure deficit (VPD) (Zeiger, 1983). The effects of temperature on stomatal behavior can vary between plants (Zeiger, 1983). Wuenscher and Kozlowski (1971) found that stomatal aperture increased as the temperature increased from 20 ℃ to 40 ℃. Meidner and Heath (1959) showed that onion (*Allium cepa*) leaves increased stomatal opening under high temperature. Wilson (1958) showed that increasing the temperature from 25 ℃ to 30 ℃ generated more stomatal opening in *Camellia japonica* L. Walker and Zelitch (1963) reported that there was a threefold change between tobacco (*Nicotiana tabacum*) leaf discs at temperatures of 10 ℃ and 30 ℃. Decreasing temperatures from 30 ℃ to 10 ℃ resulted in decreased stomatal apertures by 5 μm.

Aphalo and Jarvis (1991) showed that stomatal conductance has a better correlation with VPD. In studies with attached leaves, increasing VPD led to decreases in stomatal conductance (Schulze et al., 1980; Jarvis and Morison, 1981; Losch and Tenhunen, 1981). An increase of VPD of 1 kPa resulted in a decline in stomatal conductance from 1.2 to 0.8 in scots pine (*Pinus sylvestris* L.) (Jarvis and Morison, 1981).

Stomata in *Polypodium vulgare* L. responded to humidity independent from temperature, indicating that metabolic changes in guard cells due to humidity follow changes in stomata aperture (Losch and Tenhunen, 1981).

Previous studies tested the effects of temperature on stomata aperture in the dark and in the light (Rogers et al., 1979; Rogers et al., 1980). As observed by Losch and Tenhunen (1981), maximum stomata aperture occurred at 35 ℃ while in darkness the largest apertures occurred at 45 ℃. Rogers et al. (1981) reported that stomatal closure in the light at elevated temperature may have been caused by damage to the guard cell chloroplasts. These results support that the guard

cell chloroplasts drive stomatal opening in the light and stomatal opening in the dark is driven by oxidative phosphorylation (Zeiger, 1983).

Carbon Dioxide: CO_2 concentration and light are often linked because stomatal opening is induced by an increase in irradiance thereby activating mesophyll photosynthesis and resulting in lessened intercellular CO_2 (Jarvis and Morison, 1981). Cardon et al. (1994) showed that shifts in CO_2 levels affect shifts in stomatal conductance. Most plants stomatal aperture is inversely proportional to CO_2 concentration in both light and dark conditions. The increase in ambient or intercellular CO_2 causes stomatal closure and a decrease of CO_2 levels causes stomatal opening (Mott, 1988). Stomatal closure caused by CO_2 concentration can be noticed within seconds (Raschke, 1972). The fast response to the effect of CO_2 could be controlled by an alteration in membrane permeability, and a high acidification of the cytoplasm of the guard cell (Gepstein et al., 1982; Hsiao, 1976; Zeiger et al., 1978). Ball and Berry (1982) reported that the ratio of internal and external CO_2 concentration is crucial in controlling stomatal aperture.

The effect of CO_2 on stomatal movement mediated by guard cell chloroplasts has been explored (Meidner and Mansfield, 1968; Pallas and Dilley, 1972). CO_2 responses can differ among various species (Sharkey and Raschke, 1981b) and growing conditions can affect the capacity of phosphorylation of the guard cell chloroplasts (Melis and Harvey 1981). Inhibition of phosphorylation, CO_2 levels and stomatal closure could be positively correlated (Zeiger, 1983). The level of inhibition is reduced with light intensity at any intercellular CO_2 level and can differ with species and water stress (Zeiger, 1983). Raschke (1979) analyzed the effects of CO_2 on stomatal conductance using the CO_2 feedback loop and found slight differences for the gain, which indicated that there is a large effect on stomatal opening and the output of PAR dependent system of the guard cells regulating CO_2. Photosynthetic responses resemble stomatal conductance to irradiance (Jarvis and Morison, 1981; Zeiger and Field, 1982). There are two different responses associated with CO_2, namely CO_2 dependent responses (Mansfield et al., 1981a; Meidner and Mansfield, 1968) and CO_2 responses in the light that includes phosphorylation in the guard cells (Melis and Zeiger, 1982).

Light Effects: Light activates proton extrusion in guard cells (Gepstein et al., 1982), producing phosphorylation in the chloroplasts (Melis and Zeiger, 1982) thus opening stomatal

apertures at low light intensities (Travis and Mansfield, 1981). Guard cell protoplasts respond to light quality and intensity by swelling, which indicates reaction of guard cell photoreception (Zeiger, 1983). Different mechanisms cause light-induced stomatal opening, including blue and red light (Shimazaki et al., 2007). According to Serrano et al. (1988), the photoreceptor operation system affects the number and direction of ion transport across the guard cells. The photoreceptors responsible for this mechanism are identical to the ones responsible for photoperiod and phototropism. Ogawa et al. (1978) showed that there are two photoreceptors involved in the movement of stomata: one that is sensitive to red and far red and another that absorbs in the blue and the ultraviolet.

Stomata Action Spectra: The action spectrum is defined as the relative effectiveness of different wavelengths of light to induce a biological response (Briggs and Christie, 2002). Most researchers have constructed action spectrum for blue light response to stomatal opening (Eisinger et al., 2000; Karlsson, 1986; Ogawa et al., 1978; Skaar and Johnson 1978). These spectral characteristics were reported to be components of flavoprotein receptors (Briggs and Christie, 2002). Phot 1 and 2 not only drive phototropism, blue light induced chloroplast migration but also effectuate blue-light induced stomatal opening (Briggs and Christie, 2002). Carotenoids may be involved as receptors for stomatal opening. Frechilla et al. (2000) showed an action spectra ranging from 480 to 600 nm, red-shifted beyond the absorption range of a flavoprotein or a carotenoid, suggesting that the action spectra might reflect the formation of zeaxanthin with an absorption that ranges in the longer wavelengths. Hsiao et al. (1973) reported a full action spectrum of stomatal opening in *Vicia faba*. The study showed a peak at 420 to 460 nm, suggesting that the underlying process is not photosynthesis. At higher quantum flux density, there was red-light induced stomatal opening ranging from 600 to 680 nm. There was minimal response in the green spectrum at 540 to 560 nm, suggesting that there might be an involvement of photosynthesis. Sharkey and Raschke (1981a) reported an action spectrum on stomatal conductance in *Xanthium strumarium* using a steady state method (a leaf was irradiated to a certain wavelength at a certain intensity until the conductance remained steady and unchanged). The results showed a peak in the blue spectrum, a minor peak in the red spectrum and virtually little response in the green spectrum.

Stomatal apertures in epidermal peels that are intensity-dependent on CO_2 concentration in the air distinguish the guard cells photoresponses (Fischer, 1968). The stomatal responses are

dependent on the two photoreceptor systems in the guard cells. The effects of wavelength on stomatal opening show peaks in the blue and in the red region (Jarvis and Morison, 1981; Ogawa et al., 1979; Travis and Mansfield, 1981; Zeiger, 1980). These results showed the blue/red ratio highlights the relationship between the PAR and the guard cells blue light photosystems. Zeiger and Field (1982) showed that blue and red light have similar conductance at moderate to high intensities but blue light induces higher stomatal conductance under lower light intensities. The authors suggested the energy source driving stomatal conductance at low intensities is the PAR dependent system of the guard cell. The chloroplasts CO_2 sensitive phosphorylation mediates the PAR response (Melis and Zeiger, 1982).

Other studies include the use of monochromatic lasers, low blue light or far-red light, and high intensity red light (Zeiger and Field, 1982). An increase in stomatal conductance was observed with the addition of blue light and it was associated with an increase in intercellular CO_2 concentration. An addition of far-red light resulted in an increased photosynthesis rate and decreased intercellular CO_2 with no effect on stomatal conductance. Zeiger and Field (1982) proposed that stomatal conductance and photosynthesis respond to PAR in parallel and the coupling of photosynthesis and conductance is increased by guard cell sensitivity to intercellular CO_2 levels.

Photosynthesis and Stomatal Response: There is a relationship between stomatal conductance, photosynthesis and transpiration, although the response of stomatal conductance to these environmental signals is not clearly understood. Collatz et al. (1992) believed that these responses can be separated into at least two distinct groups: 1) the photosynthesis-dependent response which is driven by photosynthesis, and 2) the photosynthesis-independent response which induces rapid stomata opening. However, these two groups interact with each other (Collatz et al., 1992).

A metabolite response for stomata control may occur due to photosynthesis linked to stomatal conductance (Wong et al., 1979). Wong et al. (1979) stated that plants can adjust stomatal conductance according to the needs of photosynthetic apparatus for CO_2. The correlation of photosynthesis and conductance has been attributed to a direct response of guard cells to light (Sharkey and Raschke, 1981b; Wong et al., 1978).

2.2.3. Monochromatic Light Response, Mechanisms and Rates

Blue Light: Photoreceptors for blue light have been identified in the guard cells (Zeiger and Hepler, 1977). Isolated guard cell protoplasts of *Allium cepa* expanded in volume as a reaction to blue light, which proved that all elements needed for blue light-induced stomatal opening are present in the guard cells (Zeiger and Hepler, 1977). Blue light causes proton extrusion through the induction of a proton pump in the plasma membrane. Shimazaki et al. (2007) suggested that blue light acts as a signal by activating the plasma membrane H^+-ATPase. In addition, the hyperpolarization of the membrane potential drives the K^+ uptake through voltage gated K^+ channels. Zeaxanthin has been identified as the blue light photoreceptor which directly impacts the guard cell chloroplast (Zeiger and Zhu, 1998; Frechilla et al., 1999). Zeaxanthin content increased with the stomata's sensitivity to blue light and the delayed formation of zeaxanthin subdued stomatal opening regulated by blue light (Frechilla et al., 1999; Zeiger et al., 2002).
Stomatal opening has a fast and highly sensitive reaction to blue light (Shimazaki et al., 2007). The stomata opening reaches its maximum aperture typically within 20 minutes. The opening of stomata caused by blue light results in increased CO_2 uptake for photosynthesis and reduces limitations of CO_2 concentration (Zeiger et al., 1984). Leaf movement and expansion, and chloroplast accumulation increase light capture efficiency and improve photosynthetic electron transport with the mediation of phototropins (Briggs and Christie, 2002). On a quantum basis, blue light is more effective than red light in the opening of the stomata in intact leaves (Briggs, 2005). In a study by Shimazaki (2007), measurements of photosynthetic CO_2 fixation and stomata conductance were recorded in Arabidopsis (*Arabidopsis thaliana*) and rice (*Oryza sativa*) leaves. The results showed an increase in stomatal conductance, and a maximum conductance was reached within 20 minutes. Kitano and Eguchi (1992) showed that increased irradiance on cucumber (*Cucumis sativus*) seedlings at 405 W m^{-2} resulted in stabilized stomatal aperture, transpiration and water absorption within 20 minutes.

Green Light: Green light is virtually ineffective in opening the stomata compared to blue and red light. Sharkey and Raschke (1981a) conducted a study on *Xanthium strumarium* leaves that showed the effects of different wavelengths on stomata opening. Stomata did not have a strong response in the green wavelengths.

Frechilla et al. (2000) demonstrated that green light pulses could inhibit stomatal opening mediated by blue light in *Vicia faba* epidermal peels. The experiment involved irradiating lettuce with green light as a supplemental lighting to blue with a background of red light at 120 μmol $m^{-2}s^{-1}$. After being irradiated for 1 hour with red light, plants were additionally irradiated with 30 second pulses of blue light. Frechilla et al. (2000) found that green light reverses the effect of blue light on stomatal opening. The green light effect had a maximum response at 540 nm and minor peaks at 490 and 580 nm. Detached leaf peels of lettuce were put in the dark and irradiated with various light treatments. Stomata were first irradiated with blue light at 10 μmol m^{-2} s^{-1}, then the treatment was reversed by adding light between 480 and 610 nm at 10 nm intervals. At each wavelength, they determined the inhibitory effect at 5, 10 and 20 μmol m^{-2} s^{-1} (Frechilla et al., 2000). The results showed that a continuous monochromatic green light (505 – 560 nm) generated slight stomatal opening when applied alone. Green light reversed blue light induced stomatal opening and the effect of blue light was reinstated once a subsequent blue light pulse followed the green light pulse. The complete reversal was obtained with a 2:1 ratio of green and blue light, respectively.

Green light is known to impact two guard cell photoreceptors, chlorophyll and phytochrome (Frechilla et al., 2000). Sharkey and Ogawa (1987) showed that the photosynthesis-dependent stomatal opening had an action spectrum that generated minimal activity in the green region of the spectrum. Frechilla et al. (2000) stated that the reversal of blue light cannot be explained by means of photosynthetic effect due to the response of red light not being as significant as the green. Furthermore, the stomata response curve of the green region is different from the photosynthetic curve. Frechilla et al. (2000) proposed that the photoisomerization of zeaxanthin within a protein environment could explain the green light reversal. Talbott et al. (2003) confirmed that the genes phot1 and/or phot2, but not the photoreceptors, caused the blue-green reversal mechanism of stomatal opening and there are independent stomatal regulatory pathways that include NPQ1, phot1 and phot2 in the response.

Red Light: The red-light response is driven by the accumulation of K^+ ions (Hsiao, 1973) and partially by the accumulation of sugars (Olsen, 2002) produced in the guard cells from photosynthesis activity and starch degradation (Outlaw and Manchester, 1979). Studies demonstrate that stomata opening is induced by red light when high and continuous light intensities are given (Shimazaki et al., 2007). Photoreceptors of red light that control stomata are located

within the chloroplast, however, it is not clear where and how the process is activated (Shimazaki et al., 2007).

Stomatal response to red light is believed to be driven by an indirect response driven by mesophyll photosynthesis and internal CO_2 concentrations. A study conducted by Shimazaki et al. (2007) involved individual guard cells irradiated with a beam of red light. The irradiation did not induce stomatal opening which brought forth the hypothesis that the red-light response was due to the decrease in intercellular CO_2 concentration caused by mesophyll photosynthesis. The changes in intercellular CO_2 impacted the mesophyll photosynthesis which in turn triggered stomatal opening (Roelfsema and Hedrich, 2005). The red light is not only an indirect response but also a direct response driven by guard cell chloroplast to the red-light stimulus. Red light irradiated on isolated epidermis induce stomatal opening which confirms a guard cell chloroplasts response (Sharkey and Raschke, 1981a). Studies of intact leaves show that red light induces stomatal opening when the intracellular CO_2 concentration is stable (Messinger et al., 2006).

2.3. Postharvest Storage Quality

Growers struggle to maintain the quality of leafy green vegetables due to environmental factors (Prasad and Chakravorty, 2015). However, plant quality can be improved through active manipulation of lighting conditions for the production and storage of good quality vegetables (Dueck et al., 2016).

Vegetables have metabolic processes such as transpiration and respiration that continue during postharvest storage (Martinez-Romero et al., 2007). Plant storage can lead to internal and external quality losses such as increased respiration, thus increasing water loss (Toivonen and DeEll, 2002). Postharvest quality includes the following characteristics: visual scale, flavor, texture, nutritional qualities and lack of microbes (D'Souza et al., 2015). To extend postharvest quality, it is necessary to control certain factors, such as temperature, humidity, CO_2 levels and ethylene production (Kader and Rolle, 2004).

Researchers refer to senescence as the limits of the marketability or visual appearance of vegetables. Senescence is defined as a degenerative process of a cell, organ or organism; an integration of environmental or developmental signals that introduce variations in hormone fluxes that promote the active degradation of the targeted tissue (Pogson and Morrison, 2004). Cytokinins, gibberelins and some auxins slow down senescence while other phytohormones such as ethylene, abscisic acid and jasmonates increase senescence (Pogson and Morris, 2004).

Light quality increases the production of nutrients and delays senescence (Braidot et al., 2014; Costa et al., 2013). Emission of low levels of light can better sustain a crop's postharvest quality than when being stored in the dark (Braidot et al., 2014). Senescence may vary in criteria with different vegetables, and loss of chlorophyll and wilting usually indicates senescence in leafy vegetables. The loss is apparent within 24 to 48 hours at 20 ℃ when spinach leaves and broccoli are stored in the dark (Pogson and Morris, 2004). Chinese kale lasts a few days when temperatures are held at 20 ℃ or above. Mature kale stored at low temperatures in the dark can last for 10 to 14 days (Poochai et al., 1984; Wilson et al., 1988). Produce such as mandarin (*Citrus reticulate*) fruit (Yamauchi and Hashinaga, 1992) and broccoli (Yamauchi et al., 1997) turn yellow when stored at low temperatures. Light can reduce harvested plants' senescence, prevent yellowing in kiwifruit (Tombesi et al., 1993) and cabbage (Perrin, 1982) and minimize quality loss (Pogson and Morris, 2004). Noichinda et al., (2007) conducted a study testing the effect of light during storage

of Chinese kale. Mature leaves were stored at 1 ℃ with 95% relative humidity. One batch was irradiated with fluorescent tubes at 21.8 μmol m^{-2} s^{-1} and the other was stored in darkness. The results show that the total chlorophyll and ascorbic acid levels were lowest in the dark than in the light. The carotenoid, glucose, fructose and starch content were higher in leaves stored in the light. Stomatal opening was positively correlated with loss of fresh mass, showing that fluorescent light induced stomatal opening resulted in higher weight loss (Noichinda et al., 2007).

The study of the effect of LEDs on postharvest quality is relatively recent but has resulted in important findings. Efficient LED treatments for postharvest storage require a specific administration of light, dependent on light intensity, duration, photoperiod and spectral composition (Nooden and Schneider, 2004). LED light can slow ripening if plants are treated before storage. Dhakal and Baek (2014) irradiated mature tomatoes (*Solanum lycopersicum*) with 440 to 450 nm wavelengths of blue LEDs for 7 days and resulted in decreased rate of color change and firmer tomatoes compared to the non-treated tomato fruit.

The light compensation point is the light intensity at which the rate of photosynthesis is equal to the rate of respiration and can be used to determine the appropriate light intensity for the storage of leafy vegetables. Ma et al. (2014) used red LED light on broccoli (*Brassica oleracea L. var. Italica*) at an irradiance level of 50 μmol m^{-2} s^{-1} for 4 days and showed a decrease in ethylene production, higher ascorbic acid content and decreased yellowing compared to blue LED light, white LED light and a dark control. Ma et al. (2011) applied a red LED light (660 nm) treatment at 50 μmol m^{-2} s^{-1} for 6 days on Satsuma mandarins (*Citrus unshiu* Marc.) and found an increased carotenoid content when compared to blue LED light (470 nm) and a dark control. Lee et al. (2014) treated cabbage (*Brassica oleracea var. capitate*) with white, blue (436 nm), green (524 nm) and red (665 nm) LED light. All LED treatments increased total chlorophyll, vitamin C and phenolics content compared to the dark control. The green LED light treatment resulted in the highest chlorophyll content, while the blue treatment had the highest vitamin C levels, and the moisture content for all treatments showed a 5% decrease compared to the control.

Light intensities below the light compensation point may result in a net loss of sugars in fruit (Nooden and Schneider, 2004), but not under all circumstances. In certain instances, studies showed that irradiated plants with a light intensity below light compensation resulted in better storage conditions than those in the dark. Costa et al. (2013) applied a pulsed white fluorescent light on basil leaves (*Ocimum basilicum* L.) at an intensity less than the compensation point and

delayed senescence. In another study, a light intensity below the light compensation point (below 30 μmol m^{-2} s^{-1}) was irradiated on Chinese kale and recorded higher nutrients in the light than in the dark (Noichinda et al., 2007). Lester et al. (2010) tested the relationship between fresh-packaged spinach leaves irradiated with continuous light (below the compensation point) or stored in the dark. The results show that the leaves stored under continuous light had higher levels of bioactive compounds and were more nutritiously rich compared to those stored in the dark.

In a study conducted by Braidot et al. (2014), a warm white LED with a light intensity of 1.4 μmol m^{-2} s^{-1} was used for pulsed lighting on lettuce for 8 hours with 8 cycles of 1 hour pulses or 16 cycles of 30 min pulses. Both pulsed lighting resulted in increased chl *a/b* ratio above the initial ratio. Using 16 cycles of short pulses more efficiently reduced the degradation of chl *a* and *b* and resulted in slower decline of carotenoids content in lettuce, thereby delaying the loss of postharvest nutritional quality and postponed senescence. The spectral composition of the white LED light emitted included a peak at 570 nm with a width of 200 nm. Yellow light suppressed the growth of lettuce (*Latuca sativa* cv. Grand rapids), suggesting that yellow light might have decreased photosynthesis (Dougher and Bughee, 2001).

Even though ultraviolet (UV) LEDs are not part of the visible spectrum, they are suitable for postharvest applications in enhancing nutritional quality and delaying microbial growth. Kanazawa et al. (2012) reported that UV LEDs increase the production of flavonoids. Flavonoids absorb in the UV range and protect plants from UV damage. Watercress (*Nasturtium officinale* R. Br.) and garden pea sprouts (*Pisum sativum* L.) were irradiated with UV-A LED (375 nm) at a light intensity of 33 μmol m^{-2} s^{-1} for 160 min per day for 3 days then stored in the dark. After 6 days, vegetables irradiated with UV contained higher levels of quercetin-glycoside compared with those stored in the dark.

Preventing Fungal Spoilage: LEDs can be used to inhibit fungal spoilage, one cause of postharvest loss. Studies have shown that blue light treatments at 40 μmol m^{-2} s^{-1} on fungi for a period of 5 to 7 days can decrease rotten areas, mycelial growth and the formation of various fungi including *Penicillium digitatum*, *Penicillium italicum* and *Phomopsis citri*, when compared to white LED light and dark control (Alferez et al., 2012; Liao et al., 2013). Yu and Lee (2013) applied red LED light (645 nm) at 240 μmol m^{-2} s^{-1} on the surface of fruits infected by *Bacillus amyloliquefaciens JBC36* and found that red LED light increased the antifungal effect of the

bacteria. Imada et al. (2014) used a blue LED (405 nm) treatment on detached leaves of tomato plants at a light intensity of 50 W m^{-2} for 15 min h^{-1} and reported an inhibition of bacteria (*Botrytis cinerea*) growth on the leaves when treated with white light. LEDs possess the necessary properties to provide plants with the requisite light and other pulsed lighting settings for postharvest preservation. Alferez et al. (2012) irradiated tangerines (*Citrus reticulata*) with blue LED light for 3 days before inoculation with spore suspensions. A 12-hour photoperiod of blue LED light resulted in reduced mycelial growth of *P. digitatum*.

Although there has been success in using LEDs to preserve the quality of certain produce and plant parts, studies have been limited. The flexibility of LEDs need to be studied further and a better understanding needs to be reached on the impact of light on light compensation point, stomata opening, oxidative stress, senescence and protection against infection.

Chapter 3

Connecting Statement

Chapter 3, Photosynthetic response and light compensation point of 'Melody' spinach and 'Winterbor' kale, authored by Anne Sophie Rufyikiri, Mark G. Lefsrud and Valérie Orsat.

Chapter 3 covers the methodology, results and discussion of the photosynthetic response and light compensation point of spinach (*Spinacia oleracea* L.) and kale (*Brassica oleracea* L.). The chapter tackles specific parameters discussed in the literature review of this thesis by presenting photosynthetic response data in the visible spectrum under three different irradiance levels. In addition, this chapter focuses on the green spectrum of LEDs.

Abstract

Photosynthetic responses to varying light quality and light compensation point are important factors needed for optimizing plant production and food storage in controlled environments as it can help understand plant response and determine optimal growth and maintenance of postharvest storage quality. The goal for this experiment was to measure the photosynthetic response of spinach and kale treated with LEDs within the wavelengths of 400 to 700 nm to determine wavelengths that result in lower photosynthetic rates and determine the appropriate light compensation point that will induce extension of shelf life. A LI-COR 6400 portable photosynthesis measurement device with an Arabidopsis chamber was used to measure the plant photosynthetic rate. Each plant was placed in the Arabidopsis chamber, dark respiration was measured and the plant was irradiated with various wavelengths (400 – 700 nm) at 50, 100 and 150 μmol m^{-2} s^{-1} irradiance levels. The light compensation point results were as follows: for spinach, treatments of blue light (405 nm - 470 nm) were 46 – 63 μmol m^{-2} s^{-1}, green light (501 nm - 595 nm) resulted in light compensation points ranging from 53 – 80 μmol m^{-2} s^{-1} and red light (624 nm - 661 nm) resulted in light compensation points between 39 – 64 μmol m^{-2} s^{-1}. For kale, treatments of blue light resulted in light compensation points in the range of 31 – 46 μmol m^{-2} s^{-1}. Light compensation points in the green light spectrum were between 44 – 75 μmol m^{-2} s^{-1} and light compensation point in the red-light spectrum ranged from 32 – 42 μmol m^{-2} s^{-1}. The results show that the higher photosynthetic rate results in lower compensation point and the lower photosynthetic rate results in a higher light compensation point.

3. Photosynthetic Response

3.1. Introduction

LEDs have been used largely as lighting systems for horticultural facilities in recent years. LEDs are an effective lighting source due to their size, compactness, durability, narrow wavebands and cooler emitting surfaces (Massa et al., 2008). LEDs are becoming more favorable than conventional lighting sources such as halogen, fluorescent, HID and incandescent lamps (Tsao et al., 2010). Small-scale horticulture studies conducted using LEDs have shown an improvement in plant productivity and their nutritional content (Morrow, 2008; Lefsrud, 2006). The wavelengths emitted by LEDs can be precisely selected according to plant specific data, to extend postharvest quality, maximize the photosynthetic efficiency and other morphological changes (Olle and Virsile, 2013).

Light response curves provide specific data on plant light compensation point (Taiz and Zeiger, 2002). The action spectrum of plants can be gathered from the light response curve data, which is referred to as PAR. The PAR curve is the amount of light absorbed and used by various plant pigments as a function of wavelength in the visible spectrum (ranging from 400 nm to 700 nm). The appropriate administration of LED light quality and light compensation point can result in extension of shelf life quality and inhibition of growth during transport and storage (Tamulaitis et al., 2005). The light compensation point differs in species, cultivars, wavelength of light and other factors affecting respiration and photosynthesis (Ashton and Turner, 1979).

Spinach and kale are vegetables that are becoming increasingly popular and have increased interest as a year-round produce due to their nutritional benefits (Erwin and Gesick, 2017). Although data is available on the photosynthetic rate and light compensation point of leafy greens, not many studies have correlated the light compensation point of spinach and kale to LED lights at their difference wavelengths in the visible spectrum. The objective of this research was to test the effects of different light quality on spinach and kale, use the data to generate a light response curve and determine the light compensation point needed for extension of the shelf life of spinach (*Spinacia oleceara* L.) and kale (*Brassica oleceara* L.)

3.2. Material and Methods

Plant Culture: 'Winterbor' kale and 'Melody' spinach were seeded in rockwool cubes (Grodan A/S, Dk-2640, Hedehusene, Denmark) and germinated in a growth chamber (Model E15; Conviron, Winnipeg, Manitoba, Canada) under white fluorescent lights (4200 K, F72T8CW, Osram, USA) at 150 µmol m^{-2} s^{-1} irradiance, 25 ºC/25 ºC, 70% day/night temperature and relative humidity, ambient CO_2 and a daily photoperiod of 16 hours. The plants were watered weekly and with a full-strength Hoagland nutrient solution (Hoagland and Arnon, 1950).

Wavelength Testing and Photosynthetic Measurements: Measurements occurred when the 4th true leaf emerged, two weeks after germination for kale and three weeks for spinach. At this stage, the plants were treated with 14 different wavelengths of LEDs (ORBITEC, Madison, Wisconsin, USA) across the PAR spectrum (400 nm to 700 nm). The wavelengths used are as follows: 405 nm, 417 nm, 430 nm, 449 nm, 470 nm, 501 nm, 519 nm, 530 nm, 560 nm, 575 nm, 595 nm, 624 nm, 633 nm, and 661 nm. Irradiance levels tested were 50, 100, and 150 µmol m^{-2} s^{-1}; however, the 575 nm LED array could not reach an irradiance level of 150 µmol m^{-2} s^{-1} the data point used was the highest intensity obtained (140 µmol m^{-2} s^{-1}). The measurement of wavelengths and light intensities were determined using a light sensor (Spectroradiometer PS-300, Apogee Instruments, Logan, Utah, USA). The plants were tested for gas exchange using the LI-COR Arabidopsis chamber (LI-COR, Lincoln, Nebraska, USA; LI-6400XT Portable Photosynthesis System). Plants rooted in wet rockwool cubes were wrapped in parafilm for moisture retention. The test plants were placed in the Arabidopsis chamber in the dark for 10 minutes for baseline data collection. The LI-6400 controlled the relative humidity (75% +/- 3.5%), CO_2 concentration (400 ppm +/- 10ppm) and temperature (23 ℃ +/− 1℃). Light treatment with irradiance levels of 50, 100 and 150 µmol m^{-2} s^{-1} were tested (> 30 minutes) while monitoring carbon dioxide levels. Plants were placed in the dark for 10 minutes between the randomly selected wavelengths for dark respiration and to avoid carry over effects from previous measurements. After data collection, leaves were detached to calculate leaf area. A digital image was taken and Image J software (Bethesda, Maryland, USA) was used to determine leaf area and to calculate leaf-area-normalized photosynthetic rates.

Statistical Analysis: The data was analyzed using SAS (Cary, North Carolina, USA) proc GLM. The light compensation points for each wavelength were obtained using a regression analysis, estimating the relationship between the dependent variables and treatments in the experiment.

3.3. Results and Discussion

Light Compensation Points: A linear regression analysis was used to determine the light compensation point for each wavelength. For spinach, in the blue LED light spectrum, the light compensation point was 46.4 μmol m^{-2} s^{-1} at 405 nm, 51.4 μmol m^{-2} s^{-1} at 417 nm, 51.9 μmol m^{-2} s^{-1} at 430 nm, 53.2 μmol m^{-2} s^{-1} at 449 nm and 63.4 μmol m^{-2} s^{-1} at 470 nm (Figure 3.1). In the green LED light spectrum, the light compensation resulted in 65.3 μmol m^{-2} s^{-1} for 501 nm, 80.8 μmol m^{-2} s^{-1} for 519 nm, 79.7 μmol m^{-2} s^{-1} for 530 nm, 64.7 μmol m^{-2} s^{-1} for 560 nm, 68.4 μmol m^{-2} s^{-1} for 575 nm and 53.4 μmol m^{-2} s^{-1} for 595 nm (Figure 3.1). The light compensation point in the red LED light spectrum for wavelengths 624, 633 and 661 nm resulted in 54.8, 39 and 64.4 μmol m^{-2} s^{-1}, respectively (Figure 3.1).

For kale, the light compensation point in the blue LED light spectrum at 405 nm resulted in 34.9 μmol m^{-2} s^{-1}, 31.3 μmol m^{-2} s^{-1} at 417 nm, 26.5 μmol m^{-2} s^{-1} at 430 nm, 44.2 μmol m^{-2} s^{-1} at 449 nm and 46.1 μmol m^{-2} s^{-1} at 470 nm. In the green LED light spectrum, the light compensation points were 50.8 μmol m^{-2} s^{-1} at 501 nm, 62.9 μmol m^{-2} s^{-1} at 519 nm, 75.6 μmol m^{-2} s^{-1} at 530 nm, 44.1 μmol m^{-2} s^{-1} at 560 nm, 46.6 μmol m^{-2} s^{-1} at 575 nm and 28.9 μmol m^{-2} s^{-1} at 595 nm. The light compensation point in the red LED light spectrum for wavelengths 624, 633 and 661 nm resulted in 39.9 μmol m^{-2} s^{-1}, 42.9 μmol m^{-2} s^{-1} and 32.6 μmol m^{-2} s^{-1}, respectively.

The light compensation points of both spinach and kale show peaks at 530 nm to be 80 μmol m^{-2} s^{-1} but for kale other wavelengths had light compensation points 10 – 30 μmol m^{-2} s^{-1} lower than that of spinach. The difference in light compensation points for spinach and kale is similar to those reported by Erwin and Gesick (2017). 'Winterbor' kale appears to have lower compensation points due to its ability to use lower respiration rates in light limited environments. Kale can be grown and stored under lower intensity than spinach while still having a net increase in mass and maintaining better quality.

The light compensation point of spinach shows peaks at 519, 530, 575 and 661 nm (Figure 3.1). The light compensation point of kale shows peaks at 530, 575 and 633 nm (Figure 3.1). The average light compensation point of spinach was 59.8 μmol m^{-2} s^{-1} and the average light compensation point of kale was 43.4 μmol m^{-2} s^{-1}. The regression analysis of spinach and kale showed a good fit for the light compensation points. The R^2 of the linear models show a fit of 80 to 90 % (Tables 3.1 and 3.2). The data was slightly different from the work published by Erwin and Gesick (2017) showing a higher light compensation point of 73 μmol m^{-2} s^{-1} for spinach and a lower compensation point of 20 μmol m^{-2} s^{-1} for kale. In that particular study, the light compensation points were obtained using a leaf clip method with built-in LEDs (470 and 665 nm) at intensities of 0, 100, 200, 400, 600, 800 and 1000 μmol m^{-2} s^{-1}. The differences in experiments, such as leaf stage, using a leaf clip measurement method in comparison to a whole plant measurement method might account for slight nuances in data collection. Previous studies on light compensation points of spinach and kale were conducted using conventional lighting such as fluorescent, white, red and blue LED light, resulting in light compensation points ranging from 10 to 80 μmol m^{-2} s^{-1} (Glowacz et al., 2015; Erwin and Gesick, 2017; Noichinda et al., 2007). There is no data supporting light compensation point in the green LED light spectrum. Further investigations on light compensation point in the green LED light spectrum on plant photosynthetic activity would be of interest and should be further explored.

Photosynthetic Response: The action spectrum of spinach and kale resulted in peaks at 430 nm, 630 and 661 nm for spinach (Figure 3.2) and peaks at 417 nm, 430 nm and 661 for kale (Figure 3.3). The peak ranges are consistent with the McCree action spectrum (McCree et al., 1972). The blue peaks correlated with the absorption spectrum of chl *a* and *b*, lutein, β-carotene, zeaxanthin and lycopene (McCree et al., 1972). The red peaks correlated with the absorption spectrum of chl *a* and *b*, and zeaxanthin (McCree et al., 1972). In the green spectrum, the action spectrum shows low photosynthetic activity within the range of 500 to 560 nm, supporting results of the action spectrum from other species such as corn, radish, and wheat (Bulley et al., 1969; Hoover, 1937). There is a valley at 560 nm for spinach and at 530 nm for kale that may be due to a dip in absorption.

The photosynthetic rate is correlated to the light compensation point (Taiz and Zeiger, 2002). The link between photosynthetic activity and light compensation typically results in an

increase in photosynthetic rate with a corresponding decrease in the light compensation point. Wavelengths with high photosynthetic rate have a low light compensation point and wavelengths with low photosynthetic rates have a high compensation point (Figures 3.1, 3.2 and 3.3).

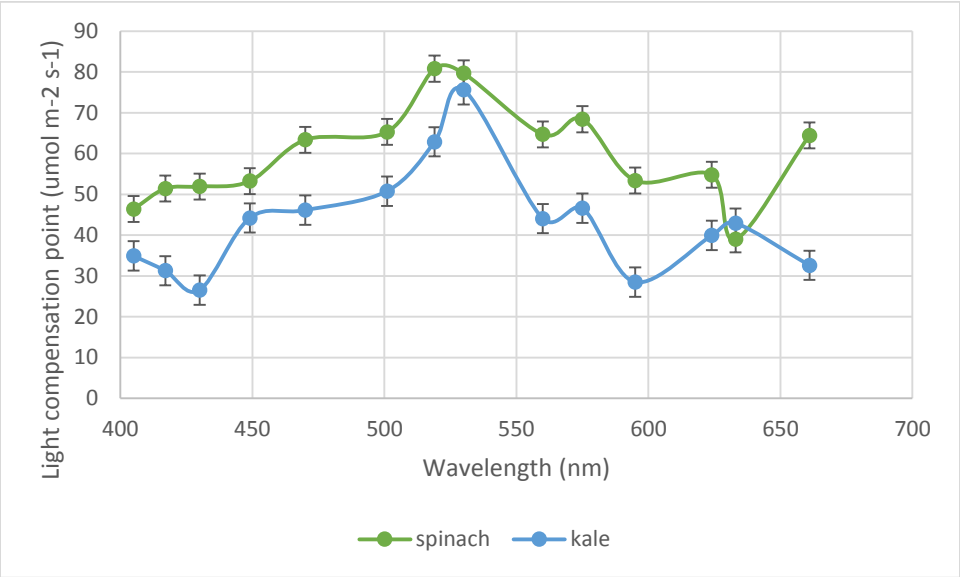

Figure 3. 1: Light Compensation Points of 'Melody' spinach (*Spinacia oleracea* L.) and 'Winterbor' kale (*Brassica oleracea* L.). Results of light compensation point of spinach. Light treatments of 14 wavelengths (405, 417, 430, 449, 470, 501, 519, 530, 560, 575, 595, 624, 633 and 661 nm) at 4 irradiance levels: 0, 50, 100 and 150 µmol m^{-2} s^{-1}. Each data point was replicated three times with different plants. The data are means of three replicates ± S.E

Table 3. 1: Light Compensation Data of 'Melody' spinach (*Spinacia oleracea* L.). Results of linear regression calculated from average relative photosynthesis of spinach seedlings from 14 LED wavelengths (405, 417, 430, 449, 470, 501, 519, 530, 560, 575, 595, 624, 633 and 661 nm) at three irradiance levels (50, 100 and 150 μmol m^{-2} s^{-1}).

Wavelength (nm)	Linear equation	R^2	Light compensation point
405	Y= 0.03x – 1.3	0.94	46.4
417	Y= 0.03x – 1.3	0.97	51.4
430	Y= 0.03x – 1.5	0.96	51.9
449	Y= 0.02x – 1.0	0.98	53.2
470	Y= 0.02x – 1.4	0.99	63.4
501	Y= 0.02x – 1.2	0.99	65.3
519	Y= 0.02x – 1.3	0.98	80.8
530	Y= 0.01x – 1.1	0.90	79.7
560	Y= 0.02x – 1.2	0.99	64.7
575	Y= 0.02x – 1.3	0.79	68.4
595	Y= 0.03x – 1.5	0.97	53.4
624	Y= 0.02x – 1.9	0.98	54.8
633	Y= 0.02x – 1.0	0.97	39
661	Y= 0.02x – 1.6	0.99	64.4

Table 3. 2: Light Compensation data of 'Winterbor' kale (*Brassica oleracea* L.). Results of linear regression calculated from average relative photosynthesis of kale seedlings from 14 LED wavelengths (405, 417, 430, 449, 470, 501, 519, 530, 560, 575, 595, 624, 633 and 661 nm) at three irradiance levels (50, 100 and 150 µmol m^{-2} s^{-1}).

Wavelength (nm)	Linear equation	R^2	Light compensation point
405	Y= 0.02x – 0.7	0.95	34.9
417	Y= 0.02x – 0.7	0.97	31.3
430	Y= 0.02x – 0.6	0.93	26.5
449	Y= 0.02x – 0.7	0.94	44.2
470	Y= 0.02x – 0.9	0.96	46.1
501	Y= 0.02x – 0.7	0.98	50.8
519	Y= 0.02x – 1.1	0.98	62.9
530	Y= 0.01x – 0.7	0.96	75.6
560	Y= 0.02x – 0.8	0.99	44.1
575	Y= 0.02x – 0.7	0.92	46.6
595	Y= 0.02x – 0.6	0.93	28.5
624	Y= 0.02x – 0.8	0.97	39.9
633	Y= 0.02x – 0.9	0.98	42.9
661	Y= 0.02x – 0.7	0.97	32.6

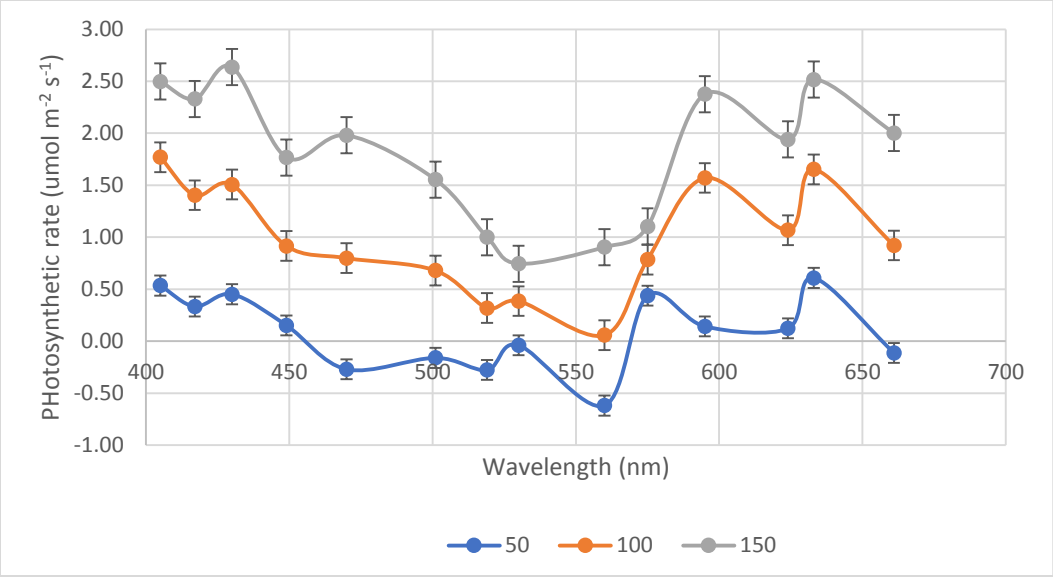

Figure 3. 2: PAR 'Melody' spinach (*Spinacia oleracea* L.). Results of average relative photosynthesis response of spinach. Light treatments of 14 wavelengths (405, 417, 430, 449, 470, 501, 519, 530, 560, 575, 595, 624, 633 and 661 nm) at three irradiance levels 50, 100 and 150 µmol m^{-2} s^{-1}.

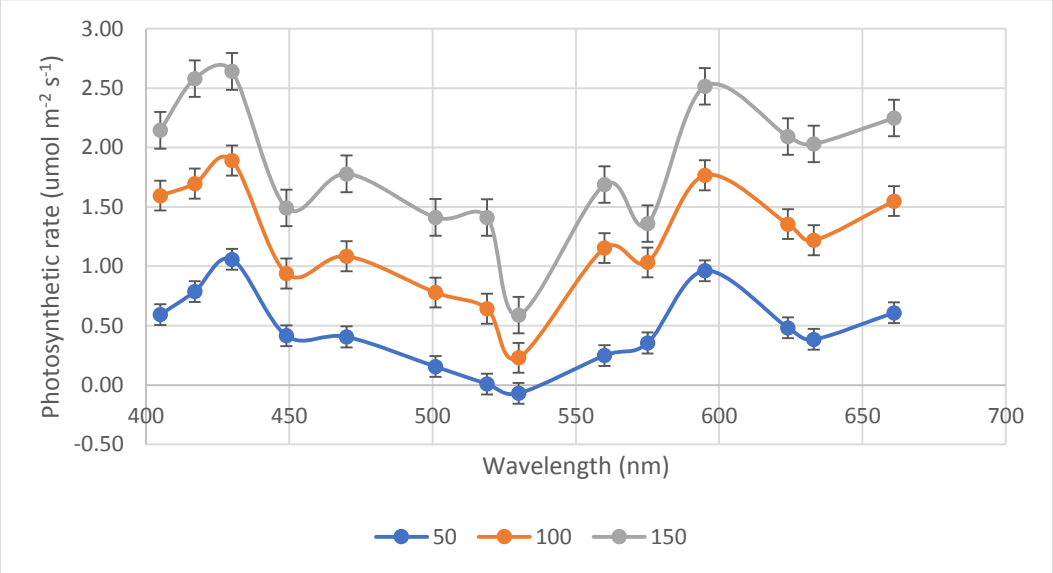

Figure 3. 3: PAR 'Winterbor' kale (*Brassica oleracea* L.). Results of average relative photosynthesis response of kale. Light treatments of 14 wavelengths (405, 417, 430, 449, 470, 501, 519, 530, 560, 575, 595, 624, 633 and 661 nm) at three irradiance levels 50, 100 and 150 µmol m^{-2} s^{-1}.

3.4. Conclusion

The light compensation points differed with species and wavelengths. The light compensation points and photosynthetic rate were typically negatively correlated. The wavelengths with high light compensation point resulted in low photosynthetic rate.

Kale light compensation point was lower than that of spinach, which suggests that kale may be stored or grown under lower irradiance levels than spinach and maintain better quality.

These results can be applied as a baseline to better understand the light compensation point data across the visible spectrum using LEDs. These results also provide data on the photosynthetic efficiency of spinach and kale that can be used for horticultural production. Based on these results, further research can be developed to determine the impact that the light compensation point of green LED light with narrow bandwidth would have on shelf life quality.

Chapter 4

Connecting Statement

Chapter 4. The effects of LEDs on stomatal opening aperture of spinach and kale authored by Anne Sophie Rufyikiri, Mark G. Lefsrud and Valérie Orsat.

Following the knowledge base developed in Chapter 3, Chapter 4 describes the methodology, results and discussion of the effects of light quality on stomatal aperture opening of spinach and kale. After determining the photosynthetic response and light compensation point, the next step was to test which wavelengths would result in low stomatal aperture opening to provide information for storage testing.

Abstract

In this experiment, stomatal aperture opening was observed in spinach (*Spinacia oleracea* L.) and kale (*Brassica oleracea* L.) treated with light emitting diodes (LED) at wavelengths from 400 to 700 nm to find wavelengths that result in reduced stomatal aperture size which should result in less transpiration. The goal of the experiment was to improve postharvest quality and extended shelf life through the control of the stomata aperture opening. Stomatal aperture size under the treatments of blue light (405 nm – 470 nm), red light (624 nm – 661 nm), and amber light (595 nm) effectively increased the stomatal opening, while green light (501 nm – 560 nm) resulted in reduced stomatal opening. These results suggest that wavelengths in the green spectrum can be used to minimize water loss and preserve postharvest quality of green leafy plants.

4. Stomata Aperture Opening

4.1. Introduction

Stomata represent a link between the plant and the outside environment. Stomata respond to changes in the environment by opening and closing which is caused by variations in the turgor of the guard cells (Hsiao, 1976). Stomatal aperture increases as the turgor pressure increases and a decline in turgor pressure decreases stomata aperture size. In general, plants have opened stomata when irradiated with light and closed stomata in the absence of light. Stomata open in the light as light initiates proton extrusion in the guard cells (Melis and Zeiger, 1982). Protoplasts in the guard cells respond to light quality and intensity by swelling, confirming guard cell photoreception reaction (Zeiger, 1983). Stomatal opening is driven by various mechanisms, including blue and red light induced responses (Shimazaki et al., 2007).

The action spectrum of stomatal opening has been reported in a group of papers, all reporting stomatal opening induced by red and blue light, while green light was not effective at stomata opening (Sharkey and Ogawa, 1987; Hsiao et al., 1976; Sharkey and Raschke, 1981). Blue and red light responses have been explored, showing zeaxanthin as the blue-light receptor (Frechilla et al., 1999) and a guard cell protoplast responding for red light (Sharkey and Raschke, 1981a).

The action spectra for blue light induced phototropism in Arabidopsis and lettuce has been observed to reach into the green spectrum (Steinitz et al., 1985). Mandoli and Briggs (1981) reported a green light response that is phytochrome-dependent. A minor sensitivity to green light driven by guard cell photosynthesis was reported by Sharkey and Ogawa (1987).

The experiment presented here determined the impact of different wavelengths on stomatal aperture opening on two different plant species, namely spinach (*Spinacia oleracea* L.) and kale (*Brassica oleracea* L.)

4.2. Material and Methods

Plant Culture: 'Winterbor' kale and 'Melody' spinach were seeded in rockwool cubes (Grodan A/S, Dk-2640, Hedehusene, Denmark) and germinated in a growth chamber (Model E15; Conviron, Winnipeg, Manitoba, Canada) under white fluorescent lights (4200 K, F72T8CW, Osram, USA) at 150 µmol m^{-2} s^{-1} irradiance, 25 ºC/25 ºC temperature, 70% relative humidity, ambient CO_2 and a daily photoperiod of 16 hours. The plants were watered weekly with a full-strength Hoagland nutrient solution (Hoagland and Arnon, 1950).

Stomatal Response: Full intact, two-week-old plants were moved from the chamber and treated with 14 LED wavelengths (405, 417, 430, 449, 470, 501, 519, 530, 560, 575, 595, 624, 633 and 661 nm) for 40 minutes to determine stomatal response using direct stomatal observation. A clear nail polish (Wildshine, Markwins Beauty products. Inc, Industry, California, USA) was painted on the upper and lower epidermis of the third true leaf in the interveinal part (~ 1 cm^2). After drying, the clear nail polish was removed using transparent tape from the leaf to extract epidermal imprints and placed on a microscope slide for counting. The stomata were observed under a microscope from three different identification fields for the upper and lower epidermis. The stomata counts were added, averaged and normalized. The counts were assigned values as open stomata at 80% aperture opening, partially open stomata at 50% aperture opening, and closed stomata at 0% aperture opening. The aperture opening was determined using Colby (2007) observation (Figure 4.1)

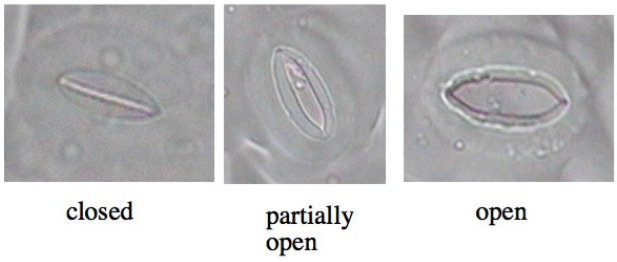

Figure 4. 1: Analysis of stomatal aperture opening Colby (2007).

Statistical Analysis: The data was analyzed using SAS (Cary, North Carolina, USA). ANOVA test was run using proc GLM at a significance level of p = 0.05. The multiple

comparisons and least square means were determined using Scheffe's test for the pairwise comparisons of plant species, wavelengths and intensity and their interaction effects.

4.3. Results and Discussion

Stomatal Response: The results show a similar action spectrum for both 'Melody' spinach and 'Winterbor' kale. The stomatal aperture openings under blue (405 – 430 nm) and red (624 – 661 nm) LED light showed higher stomatal aperture opening, while the stomata aperture opening was lower under the green LED light (500 – 560 nm).

For spinach, Figure 4.2 shows an average aperture opening of 45 to 57% at 405 to 430 nm, 595 nm, 633 nm, and 661 nm at 50, 100 and 150 μmol m^{-2} s^{-1} irradiance levels. While an average aperture opening of 28% to 33% was observed at 501 nm and 36 to 42 % was observed at 500 to 530 nm, respectively. The aperture opening of 560 nm and 575 nm were 26% to 45% for 50, 100 and 150 μmol m^{-2} s^{-1} (Figure 4.2).

For kale seedlings, the results show a 46 to 57% aperture opening at wavelengths ranging from 405 to 470 nm for all irradiance levels (Figure 4.3). In the green light spectrum, the aperture opening ranged from 26 to 37% for all irradiances. In the red-light spectrum, the aperture opening was between 45 and 57% for all irradiances (Figure 4.3).

Scheffe's test for multiple comparisons showed that wavelength treatments varied, some wavelengths were statistically different from one another and others were not statistically different. Wavelengths 501, 519, 530, 560 and 575 nm were statistically different from wavelengths 405, 417, 430, 449 and 470 nm as well as wavelengths 595, 624, 633 and 661 nm. Wavelength 575 nm was not statistically different from 624 nm. When the data was analyzed by SAS proc GLM, the procedure showed a model with a mean of 44.6 for stomatal opening aperture, a mean square standard error of 4.9 and an R^2 of 0.79 with a 79% fit of the model. Wavelength and irradiance were statistically significant for both spinach and kale (Table 4.1). Multiple comparison tests for the intensity effect in the model showed least square means of 42.4, 45.1 and 46.3 for 50, 100 and 150 μmol m^{-2} s^{-1}, respectively with a standard error of 0.5. The intensity effect in the model showed a statistical significance ($p < 0.05$) for 50 and 100 μmol m^{-2} s^{-1} as well as 50 and 150 μmol m^{-2} s^{-1} but there was no significant difference between 100 and 150 μmol m^{-2} s^{-1}. The irradiance levels did not have a large effect on stomatal aperture opening irrespective of plant type. Higher

irradiance levels (> 150 μmol m^{-2} s^{-1} and < 1500 μmol m^{-2} s^{-1}) may induce larger differences in stomatal aperture opening as demonstrated in the work of Sharkey and Raschke (1981a). In the blue spectrum, at low and high intensities, stomatal response is still sensitive to blue light unlike red light which has a stronger stomatal response at higher intensities (Zeiger and Field, 1982). Green light would require significantly higher irradiance levels to induce stomatal response as seen in the blue and red regions (Sharkey and Raschke, 1981a). The interaction effect of wavelength and intensity were statistically significant ($p < 0.05$), the interaction effect of plant species and intensity were not statistically significant. The interaction effect of plant, wavelength and intensity were not statistically significant (Table 4.1). The interaction effects that were not statistically significant were removed from the statistical model (Table 4.1).

The differences between the aperture opening for spinach and kale in the blue spectrum were at 405 nm at 50 μmol m^{-2} s^{-1}. The aperture opening percentage was lower for kale than it was for spinach (Figures 4.2 and 4.3). The least square means for spinach was slightly higher than that of kale at 44.71 and 44.45, respectively with a standard error of 0.44. The results show that the stomatal opening apertures were slightly lower in kale than spinach which may be due to its capacity to use lower rates of respiration at lower light intensities.

In the green spectrum, the lowest aperture opening percentage occurred at 560 nm for kale at all irradiances (Figure 4.3), at 560 nm for 50 and 100 μmol m^{-2} s^{-1} and at 575 nm for 50 μmol m^{-2} s^{-1} for spinach (Figure 4.2). For spinach, 519 nm at 50 μmol m^{-2} s^{-1} showed a peak (Figure 4.2) but showed a low aperture opening for kale (Figure 4.3). These results show a difference in response for spinach and kale although the difference in stomatal opening aperture for spinach and kale was not statistically significant. The nuances in peaks were only limited to a few wavelengths.

These results support studies on action spectrum of stomatal opening by Sharkey and Raschke (1981a). Blue light induced the highest percentage of aperture opening, compared to red and green (Sharkey and Raschke, 1981a; Ogawa et al., 1978; Hsiao et al., 1973). Red light resulted in stomatal opening higher than green light (Sharkey and Ogawa, 1987). The results in the green spectrum resemble work done by Folta and Maruhnich (2007) stating that green light does not induce stomatal opening as seen under red and blue light.

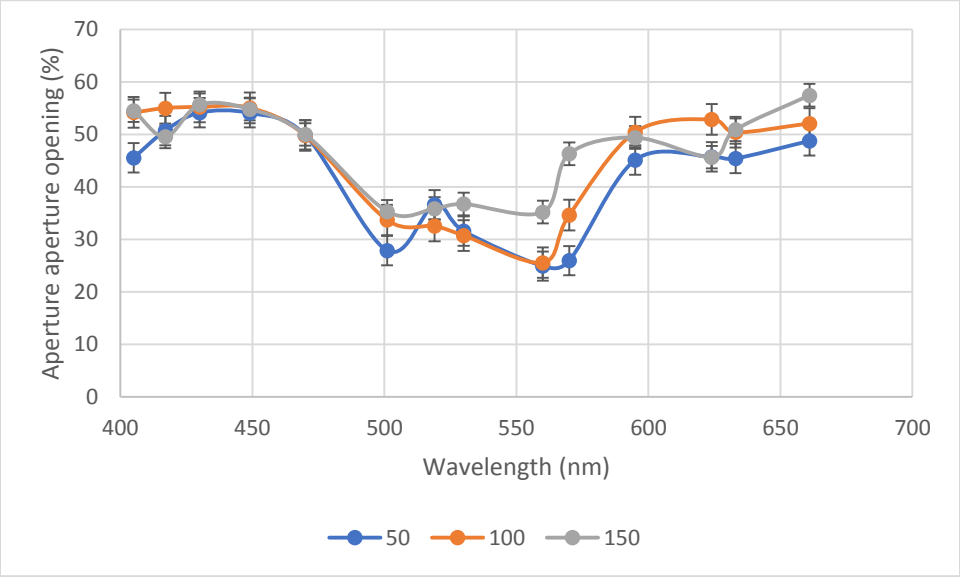

Figure 4. 2: Stomatal Aperture Opening of 'Melody' spinach (*Spinacia oleracea* L.). The average percentage of stomatal aperture opening of kale seedlings at an irradiance level of 50, 100 and 150 µmol m^{-2} s^{-1} from 14 LED wavelengths (405, 417, 430, 449, 470, 501, 519, 530, 560, 575, 624, 633 and 661 nm). The data are means of three replicates ± S.E.

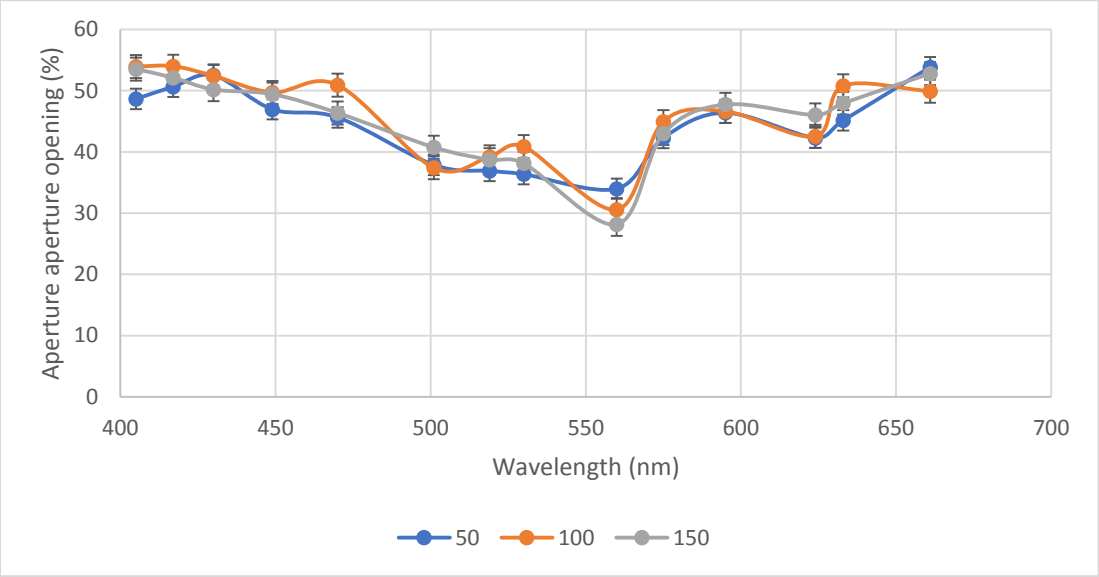

Figure 4. 3: Stomatal Average Aperture Opening of 'Winterbor' kale (*Brassica oleracea* L.). The average percentage of stomatal aperture opening of kale seedlings at an irradiance level of 50, 100 and 150 µmol m^{-2} s^{-1} light from 14 LED wavelengths (405, 417, 430, 449, 470, 501, 519, 530, 560, 575, 595, 624, 633, and 661nm). The data are means of three replicates ± S.E

Table 4. 1: Statistical Analysis of 'Melody' spinach (Spinacia oleracea L.) and 'Winterbor' kale (Brassica oleracea L.). Results of SAS proc GLM test of significance at α = 0.05.

Source	DF	Type III SS	MS	F value	Pr > F
Plant	1	4.32	4.32	0.18	0.67
Wavelength	13	14978.08	1152.16	48.08	<.0001
Intensity	2	661.17	330.58	13.79	<.0001
Plant*wavelength	13	940.62	72.36	3.02	0.0005
Plant*intensity	2	54.93	27.46	1.15	0.32
Wavelength*intensity	26	584.83	22.49	0.94	0.55

4.4. Conclusion

The stomata response varied with plant species, wavelength and intensity. The results obtained in this experiment are consistent with the data previously reported on the action spectrum of stomata opening (Sharkey and Ogawa, 1987; Hsiao, 1976).

As hypothesized, green LED wavelengths (500 – 560 nm) had lower aperture opening. The wavelengths in the green spectrum were similar in stomatal response thus requiring more testing to have a better understanding of the link between stomatal opening, moisture loss and their influence on shelf life quality.

Further work needs to be performed to address the different effects of green LED light, specifically in the 530 to 565 nm range, on stomata opening aperture.

Chapter 5

Connecting Statement

Chapter 5, The use of light-emitting diodes (LEDs) for the shelf life extension of spinach and kale, authored by Anne Sophie Rufyikiri, Mark G. Lefsrud and Valérie Orsat

Previous chapters reported on photosynthetic rate (light compensation point) and stomatal aperture opening as impacted by different wavelengths of light. The results provided information on wavelengths with low photosynthetic response, reduced stomata opening aperture and the corresponding light compensation points. Wavelengths were selected for plant shelf life testing based on data recorded in Chapters 3 and 4. Chapter 5 describes the methodology, results and discussion of the plant storage shelf life testing of spinach (*Spinacia oleracea* L.) and kale (*Brassica oleracea* L.) treated with 4 different treatments, dark (control), white fluorescent light, 500 nm and 560 nm green LED light.

Abstract

About 30% to 40% of fresh produce is never eaten due to low quality or poor aesthetics which is the result of a loss of turgor pressure in the plant, yellowing of leaves and senescence (leaf death and leaf drop). Storing leafy green vegetables in darkness is known to result in senescence, while exposure to light reverses the initial phase of senescence. In this study, the effects of different light emitting diode (LED) wavelengths were tested on leafy vegetables during storage to investigate the ability to extend the shelf life of the plants for retail sales. Cut leaves of mature spinach (*Spinacia oleracea* L.) and kale (*Brassica oleracea* L.) (without roots) were placed in trays enclosed in boxes under dark, white fluorescent, 500 nm LED and 560 nm LED light treatments. Initial mass and final mass were recorded before and after treatment to determine moisture content. The leaves were observed daily and their quality was visually assessed on a scale from 1 to 5 (5 being the best). The plants treated with 560 nm LED light resulted in a better visual quality score for both plants with a score of 3 at the end of the 5-day testing, with a moisture loss of 40% for kale and 41% for spinach. White fluorescent and 500 nm LED light resulted in a visual score of 2 at the end of the 5 days of testing and resulted in a moisture loss of 44% (fluorescent, spinach), 45% (500 nm, spinach), 46% (500 nm, kale), and 50% (fluorescent, kale). The control treatment resulted in a visual score of 1 at day 4 and a moisture loss of over 50% for both spinach and kale. The results of this study show that daily irradiation (12-hour photoperiod) of green LED wavelengths (500 nm and 560 nm) with 50-60 μmol m^{-2} s^{-1} during storage at 20℃ is an effective treatment to extend shelf life quality of spinach and kale.

5. Shelf Life Quality

5.1. Introduction

Processes such as transpiration and respiration continue during the postharvest storage of vegetables (Martinez-Romero et al., 2007) which can lead to quality losses due to high respiration resulting in increasing water loss (Toivonen and DeEll, 2002). Storage in the dark or under very low light can cause senescence (leaf yellowing and drop) due to insufficient water and nutrients as well as a deficiency of photosynthesis (Ella et al., 2003). Senescence is a degenerative process of a plant that involves the degradation of chlorophyll and protein, and the accumulation of ammonium in the leaves (Rolny et al., 2011). For leafy vegetables, senescence is indicated by wilting and the loss of chlorophyll. The senescence process of leaves kept in the dark can be reversed through the application of light (Zavaleta-Mancera et al., 1999).

Low intensity light has been reported to improve the postharvest quality of plants better than those stored in the dark (Braidot et al., 2014). Light has been used to prevent senescence in cabbage (Perrin, 1982) and broccoli (Zhan et al., 2012). Lester et al. (2010) tested the effects of continuous light on spinach leaves and compared it to leaves stored in the dark. Their results showed that the leaves stored under continuous light had higher levels of bioactive compounds and were more nutritiously rich.

Light treatments on vegetables during storage have resulted in an increase in physiological activity (Sanz et al., 2009) and increased respiration of freshly cut vegetables (Ayala et al., 2009). An appropriate application of light intensity, duration, photoperiod and spectral composition plays a crucial role for the preservation of postharvest quality (Nooden and Schneider, 2004). The aim of this paper was to test different treatments of light to extend the shelf life quality of spinach and kale.

5.2. Material and Methods

Shelf Life Testing: Standard commercial six to eight-week-old kale (Winterbor; IGA Atwater, Montreal, Quebec, Canada) and spinach (Melody; Adonis Atwater, Montreal, Quebec, Canada) were purchased for this experiment. The kale and spinach purchased at the retail stores were from different farms, but each species came from the same farm for each replicate. Only newly displayed produce was selected for the treatment experiment. The treatments included: darkness (control), standard white fluorescent light (50 µmol m^{-2} s^{-1}), 500 nm (55 µmol m^{-2} s^{-1}) and 560 nm (55 µmol m^{-2} s^{-1}) LED wavelengths. The plants were placed in boxes (length: 64 cm, width: 40 cm, height: 40 cm) lined with black plastic and stored for 6 days at a temperature of 20 ^{o}C, humidity of 50% and a daily photoperiod of 12 hours. Each treatment contained three mature plants of kale and three mature plants of spinach cut just above the roots. The fresh mass of each plant was recorded before and after each treatment. The difference between the initial fresh mass and the final fresh mass was divided by the initial fresh mass to obtain the percentage of moisture loss after 4 days of treatment. The fresh mass was used to obtain moisture content on a fresh mass basis. The visual assessment scale was determined using three separate individuals to observe and record their responses for the leaves on a scale of 1 – 5 as stated by Kader and Cantwell (2010). The plant quality scale is shown in Figure 5.1. After treatment, each plant was dried in an isotemp oven (Fischer Scientific Isotemp, Charlottesville, Virginia, USA) at 40 ^{o}C for three days and dry mass was recorded.

Figure 5. 1: Visual scale for scoring visual quality assessment of 'Winterbor' Kale (*Brassica oleracea* L.) and 'Melody' spinach (*Spinacia oleracea* L.) (Kader and Cantwell, 2010).

Statistical Analysis: The data was analyzed using SAS (Cary, North Carolina, USA). An ANOVA test was run using proc GLM at a significance level of $p = 0.05$. The multiple comparisons and least square means were determined using Scheffe's test to compare plant species, light treatments and the interaction effects of these factors.

5.3. Results and Discussion

Moisture Content and Loss: Mature detached leaves of spinach lost moisture and visual quality over the course of the 5-day storage. For spinach, the initial fresh mass, final fresh mass and dry mass were 21.6 ± 4.5 g, 12.6 ± 1.6 g and 3.5 ± 1.5 g under the 560 nm treatment, respectively. Under the 500 nm treatment, the results show an initial fresh mass of 21.1 ± 3.3 g and a final fresh mass of 11.5 ± 1.9 g, and a dry mass of 3.9 ± 2.2 g. Under the fluorescent light treatment,

the results show an initial fresh mass of 20.9 ± 6.6 g, final fresh mass of 11.5 ± 3.2 g, and final dry mass of 3.8 ±1.9 g. In the dark (control) treatment, the initial fresh mass, final fresh mass and dry mass were 20.7 ± 4.5 g and 10.1 ± 4.3 g, 3.5 ± 1.7 g, respectively (Table 5.1). Spinach moisture content showed 71.4 ± 0.1 % at 560 nm, 83.5 ± 0.02 % at 500 nm, 81.7 ± 0.1 % under fluorescent light and 105 ± 0.3 % for dark (Table 5.1). The moisture content measurements resulted in losses of 41 ± 3.5 % under 560 nm treatment, 45 ± 0.6 % under 500 nm, 44 ± 3.8 % under fluorescent treatment, and 52 ± 5.6 % for the dark treatment (Figure 5.2). Spinach has a short postharvest life caused by an inability to preserve a state of homeostasis after detachment (Pogson and Morris, 2004). The moisture loss obtained was higher in the dark than it was for the fluorescent light. These results are consistent with studies by Braidot et al. (2014) showing that light treatments lead to better quality. The moisture loss under the fluorescent light compared with both the 500 nm and 560 LED treatments differed slightly, showing a lower moisture loss under the 560 nm treatment compared to the 500 nm and fluorescent light. We were unable to find any studies that have compared the differences between green LED light and fluorescent light but there are studies showing differences between blue and red LED light and fluorescent light for the storage of broccoli (Wilson et al., 1998). The data showed that both red and blue LED lights preserved the quality better than fluorescent light. Other studies compared the effect of white, red, green and blue LEDs on storage and reported an increase in chlorophyll content of cabbage under green LED irradiation (Lee et al., 2014).

For kale, the effects on shelf life quality assessments differed among treatments. The initial fresh mass, final fresh mass, and dry mass were 42.2 ± 4.2 g, 25.4 ±1.1 g, and 6.8 ± 4.2 g under the 560 nm treatment, respectively. Under the 500 nm treatment, the results show an initial fresh mass of 38.2 ± 15.4 g, final fresh mass of 20.9 ± 7.8 g, and dry mass of 6.9 ± 3.7 g. Under the fluorescent light treatment, the results were 34.0 ± 8.0 g of initial fresh mass, 16.9 ± 2.8 g of final fresh mass, and 5.7 ± 2.4 g of dry mass, respectively and 32.6 ± 1.7 g, 15.2 ± 4.5 g, and 5.2 ± 2.9 g in the darkness (Table 5.1). Kale resulted in fresh basis moisture content of 66.1 ± 0.1 % at 560 nm, 83.8 ± 0.2 % at 500 nm, 102 ± 0.2 % under fluorescent and 122 ± 0.4 % in the dark (Table 5.1). The moisture loss reported under the 560 nm treatment was 40 ± 2.9 %, while the 500 nm treatment had 46 ± 6.0 % moisture loss, fluorescent treatment had 50 ±4.9 % loss and dark treatment had 54 ± 8.3 % moisture loss (Figure 5.2). Fluorescent light had lower moisture losses compared to the dark treatment. It supports recent studies showing that low levels of

fluorescent light maintain better quality than storage in the dark (Noichinda et al., 2007). Among other treatments, 560 nm resulted in 15% lower moisture loss compared to the produce stored in the dark and nearly 10% lower moisture loss compared to leaves stored under the fluorescent light and 500 nm LED light treatments (Figure 5.2). The 560 nm treatment was significant ($p > 0.05$) in comparison with the dark treatment. However, when comparing fluorescent and 500 nm to the dark treatment, the change in moisture was not considered significant ($p > 0.05$).

The SAS GLM procedure for moisture loss resulted in a mean measured value of 46.8, mean standard error of 4.8 and a R^2 of 0.52. The effect of plant type was not statistically significant.

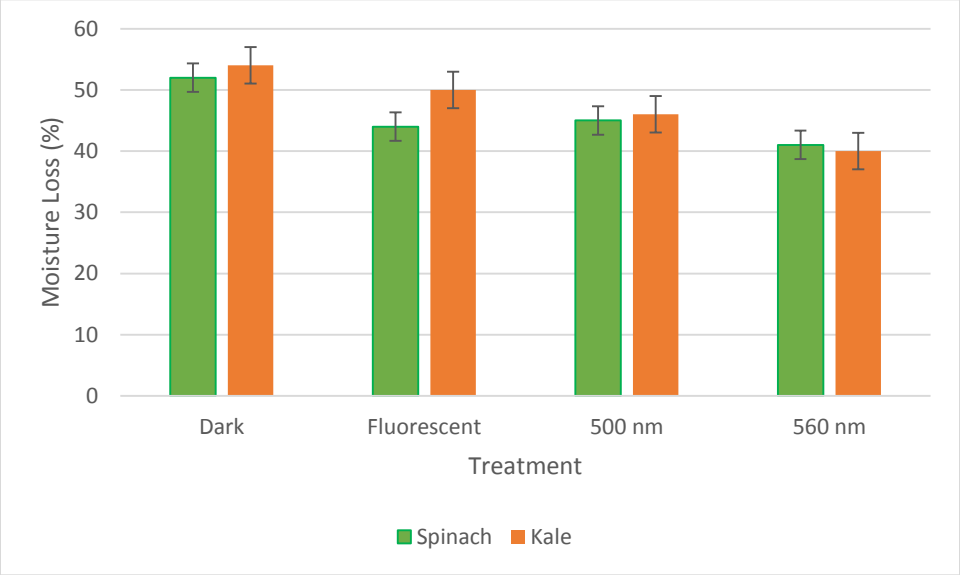

Figure 5. 2: The Moisture Loss of 'Melody' spinach (*Spinacia oleracea* L.) and 'Winterbor' kale (*Brassica oleracea* L.). The results of average moisture loss of spinach and kale after 4 days of storage under four treatments: dark, fluorescent, 500 nm and 560 nm.

Table 5. 1: The Fresh Mass, Dry Mass and Moisture Content of 'Melody' spinach (*Spinacia oleracea* L.) and 'Winterbor' kale (*Brassica oleracea* L.). Results of initial fresh mass, final fresh mass, dry mass and moisture contents (fresh basis) of spinach and kale under four treatments: dark, fluorescent (FL), 500 nm LED, and 560 nm LED.

	Initial fresh mass (g)		Final fresh mass (g)		Dry mass (g)		Moisture loss (%)		Moisture content (%)	
	spinach	kale	spinach	kale	spinach	kale	spinach	kale	spinach	kale
Dark	20.7 ± 4.5	32.6 ± 1.7	10.1 ± 4.3	15.2 ± 4.5	3.5 ± 1.7	5.2 ± 2.9	52 ± 5.6	54 ± 8.3	105 ± 0.3	122 ± 0.4
FL	20.9 ± 6.6	34 ± 8.0	11.5 ± 3.2	16.9 ± 2.8	3.8 ± 1.9	5.7 ± 2.4	44 ± 3.8	50 ± 4.9	81.7 ± 0.1	102 ± 0.2
500nm	21.1 ± 3.3	38.2 ± 15.4	11.5 ± 1.9	20.9 ± 7.8	3.9 ± 2.2	6.9 ± 3.7	45 ± 0.6	46 ± 6.0	83.5 ± 0.02	83.8 ± 0.2
560nm	21.6 ± 4.5	42.2 ± 4.2	12.6 ± 1.6	25.4 ± 1.1	3.5 ± 1.5	6.8 ± 4.2	41 ± 3.5	40 ± 2.9	71.4 ± 0.1	66.1 ± 0.1

Table 5. 2: Moisture Loss Statistical Analysis of 'Melody' spinach (*Spinacia oleracea* L.) and 'Winterbor' kale (*Brassica oleracea* L.). Results of SAS proc GLM test of significance of $\alpha = 0.05$.

Source	DF	Type III SS	MS	F value	Pr > F
Plant	1	22.04	22.04	0.95	0.34
Treatment	3	449.46	149.82	6.46	0.003

Visual Assessment: For the visual assessment of quality of spinach, fluorescent, 500 nm LED and 560 nm LED had similar responses. The visual quality at 560 nm LED and fluorescent lights maintained a score of 5 in the first 2 days, then decreased from day 3 to day 5 (Figure 5.3). On day 2, the spinach leaves under the dark treatment had lost an observable amount of moisture and started to wilt. On day 3, the fluorescent, 500 nm and 560 nm light treatments started developing a dark brownish color on the leaves and lost firmness. After 4 days of storage at room

temperature, the fluorescent, 500 nm and 560 nm LED light treated spinach were better at preserving its quality than the dark, as the former had slightly firmer leaves (Figure 5.5). When stored in the dark, the quality of spinach decreased rapidly and deteriorated by day 2 while plants that had a light treatment maintained quality until day 4. The results show that light helped preserve the quality of leaves better than darkness and is similar to the results of Lester et al. (2010).

The visual assessment which monitored quality throughout the treatment showed a better visual quality score for kale under the 560 nm treatment. The 560 nm light was better at extending the shelf life by 2 days compared to fluorescent and 500 nm light, and by 3 days compared to the dark treatment (control). On day 1, the 500 nm LED, 560 nm LED and fluorescent light treatments maintained a quality score of 5 with the leaves still green and firm. The control lost moisture on day 1 and the leaves started yellowing on the edges. On days 2 and 3, the 500 nm LED, 560 nm LED and fluorescent light treatments had a leaf in each treatment starting to yellow on the edges with noticeable loss of moisture. On day 3, the visual quality of 500 nm and fluorescent treatments decreased below 4 (Figure 5.4) and showed increased yellowing on the leaves (Figure 5.5). Kale under the 560 nm LED light treatment could have been stored for another day or two maintaining its visual quality above a score of 2 (Figure 5.4).

All light treatments were significant ($p < 0.05$) when compared to the dark treatment (Table 5.2), supporting the work of Noichinda et al., 2007 and Lester et al., 2010 stating that continuous light improved postharvest quality of spinach and kale. These results support reported work by Pogson and Morrison (2004) stating that at 20 ℃, spinach and kale last 4 days in storage in the light and 2 days in the dark. The visual quality of kale under the dark treatment decreased rapidly below marketability (visual assessment of > 2) by day 4 which may be due to senescence caused by insufficient photosynthetic activity in the dark. The 560 nm treatment was significant compared to the fluorescent treatment ($p < 0.05$). However, the comparison between 500 nm and 560 nm LEDs was considered not significant ($p > 0.05$).

The ANOVA significance test showed that the plant, days and treatment were significant (Table 5.3), confirming that plant types under different treatments showed responses that varied daily. The Scheffe's multiple comparisons adjustment showed a significant difference for the plant interaction. The least square means of kale was significantly higher than that of spinach, 3.75 and 3.40, respectively. The results show that kale had better visual quality compared to spinach

throughout the 5-day storage which may be due to a lower compensation point and stomatal opening aperture in kale than in spinach as reported in Chapters 3 and 4.

The interaction between the days of storage were all statistically significant. The least square means showed results of 4.9, 4.1, 3.0 and 2.3 for day 1, 2, 3 and 4, respectively and a standard error of 0.1. Room temperature storage resulted in increased moisture loss and faster loss of visual quality; the visual differences can be seen during the 5-day storage.

Shelf life extension requires light that induces stomatal aperture closure, to minimize moisture loss and this is supported by previous studies that have investigated the effects of light during postharvest storage (Lester et al., 2010; Noichinda et al., 2007; Toledo et al., 2003). Previous studies showed that continuous low intensity light (26 – 30 μmol m^{-2} s^{-1}) prevented loss of quality in kale (Noichinda et al., 2007) and spinach (Toledo et al., 2003; Lester et al., 2010). The intensity used in this experiment was higher (55 μmol m^{-2} s^{-1}), based on the results of the light compensation data obtained in Chapter 3. Higher light compensation point may not have had a significant impact on these results. Studies have shown that lower compensation point may still prevent senescence (Costa et al., 2013; Braidot et al., 2014), and the effect of higher red LED light compensation point (50 μmol m^{-2} s^{-1}) on plant storage, resulted in an increase in nutritional quality and a decrease of ethylene production in broccoli (Ma et al., 2014). Further testing needs to be done to determine the effects of higher compensation point of green LEDs on the storage of spinach and kale, specifically.

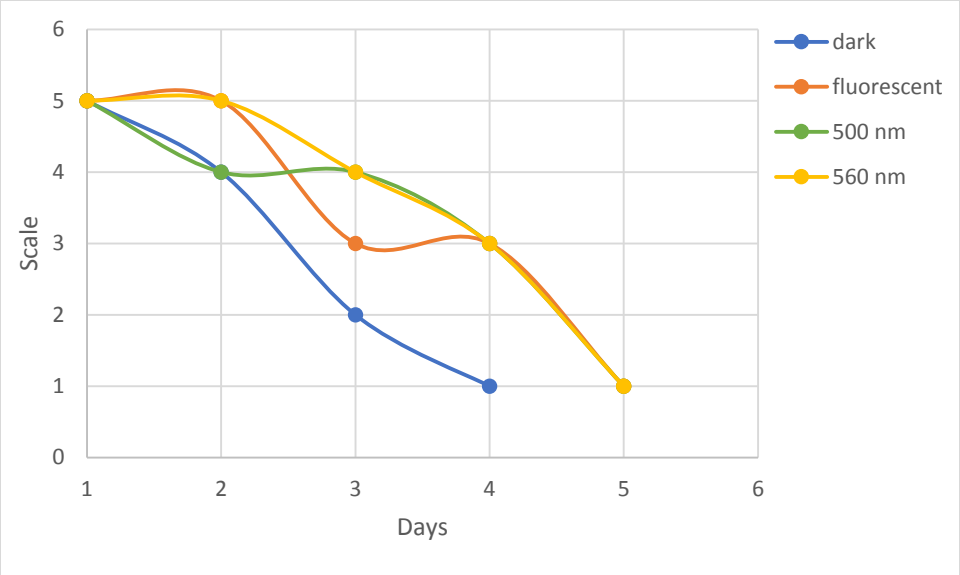

Figure 5. 3: Visual Score of 'Melody' spinach (*Spinacia oleracea* L.). The results of the visual quality scale for spinach are presented over a period of 5 days. The dark treatment is lacking data on day 5 because the quality had deteriorated beyond measurable levels.

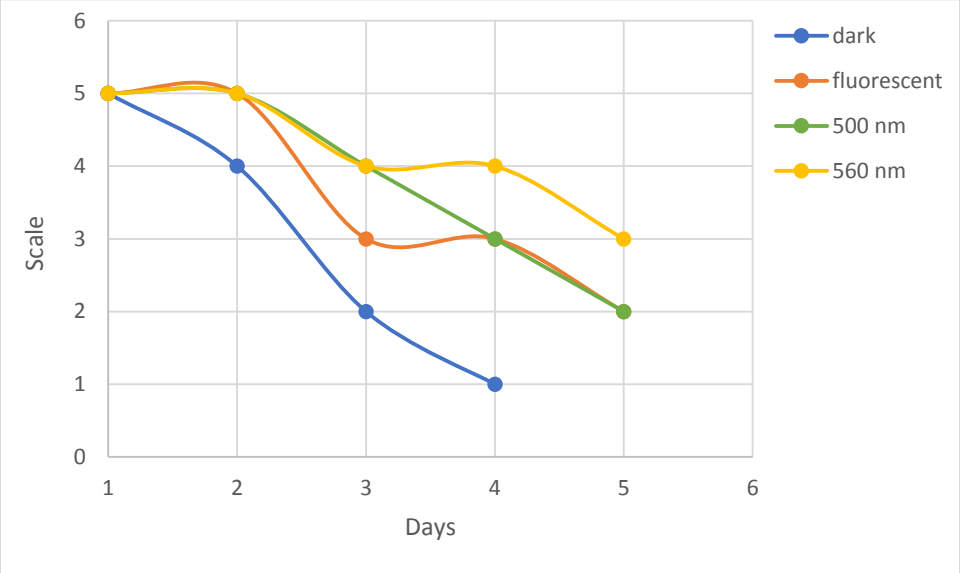

Figure 5. 4: Visual Score of 'Winterbor' kale (*Brassica oleracea* L.). The results of visual quality scale for kale are presented for a period of 5 days. The dark treatment is lacking data on day 5 because the quality had deteriorated beyond measurable levels.

Table 5. 3: Visual Scale Statistical Analysis of 'Melody' spinach (*Spinacia oleracea* L.) and 'Winterbor' kale (*Brassica oleracea* L.). Results of SAS proc GLM test of significance of α=0.05.

Source	DF	Type III SS	MS	F value	Pr > F
Plant	1	3.01	3.01	12.41	0.0007
Days	3	95.28	31.76	130.98	<.0001
Treatment	3	21.61	7.02	29.71	<.0001
Days*treatment	9	4.43	0.49	2.03	0.0467

Figure 5. 5: The shelf life testing of 'Melody' spinach (*Spinacia oleracea* L.) and 'Winterbor' kale (*Brassica oleracea* L.). The results after 4 days of storage: dark, fluorescent, 500 nm and 560 nm.

5.4. Conclusion

The data obtained in this experiment showed that LED wavelengths in the green spectrum may be the preferred wavelength range for extension of shelf life of spinach and kale.

Treatments of 500 nm and 560 nm LEDs maintained better visual quality and reduced moisture loss. The results were consistent with studies showing the efficiency of LEDs for the extension of postharvest quality compared to dark and fluorescent treatments.

Based on these results, the long-term use of green LEDs to extend the shelf life quality of leafy vegetables can be beneficial for postharvest quality and food security.

6. General Summary

6.1. General Conclusion

The objective of this research was to test the effects of LEDs on stomatal aperture opening, photosynthetic response, light compensation point and use the data to determine an LED wavelength that can extend the shelf life of spinach and kale. Current literature shows that LEDs can be used to reduce food spoilage and enhance nutritional quality thereby improving the postharvest quality of fresh produce. LEDs are appropriate for postharvest storage due to the LEDs low radiation heat emission and increased efficiency at low temperatures (D'Souza et al., 2015).

Gas exchange of 'Melody' spinach and 'Winterbor' kale was monitored using observational stomatal aperture and the LI-COR portable photosynthesis system (LI-COR, Lincoln, Nebraska, USA; LI-6400XT). The LI-COR provides a leaf clip with built-in red (665 nm) and blue (470 nm) LEDs. In this experiment, it was important to have the option to test more wavelengths, for that reason, two to three-week-old live plants were used to ensure the plants could fit in the Arabidopsis chamber. Light treatments were tested across the visible spectrum to determine which wavelengths effectively close the stomata and extend plant shelf-life during storage.

The first set of data collected was the photosynthetic response measurements of spinach and kale treated with LED wavelengths from 400-700 nm at 50, 100 and 150 µmol m^{-2} s^{-1}. The results showed a photosynthetic response that decreased in the green spectrum (500 nm – 560 nm). The light compensation points of the green LED wavelengths 500 nm and 560 nm were 45 – 65 µmol m^{-2} s^{-1} for both spinach and kale.

The second set of data was the stomatal aperture size measurements of spinach and kale treated with LED wavelengths from 400 – 700 nm at 50, 100 and 150 µmol m^{-2} s^{-1}. These results corresponded with previous research (Sharkey and Raschke 1981a) showing the effect of light quality on stomatal opening. There is an increased stomatal opening response in the blue region from 405 – 470 nm, moderate stomatal opening response in the red region (624 – 661 nm) and a reduced stomatal aperture opening size in the green spectrum (500 – 560 nm).

The third experiment combined the knowledge obtained from the first two experiments and performed a postharvest quality test of spinach and kale treated with green LED wavelengths at 500 nm and 560 nm, white fluorescent and dark as a control. The 560 nm treatment was successful at extending shelf life and postharvest quality of spinach and kale by an extra 24 hours compared to the other treatments. The 560 nm treatment resulted in 10 – 15 % lower moisture loss compared to the control and other treatments. The visual assessment of the 560 nm treatment showed a score of 2 at the end of day 4 for spinach and a score of 3 at the end of day 5 for kale, compared to the 500 nm and fluorescent treatment showing a score of 2 at the end of day 4 for spinach and a score of 2 at the end of day 5 for kale.

6.2. Future Suggested Work

In this experiment, the 560 nm treatment provided the best measured results for maintaining shelf life quality for spinach and kale. 560 nm resulted in extended postharvest quality for an extra day under room temperature storage conditions but further testing needs to be done. Recent studies have used live plants for photosynthetic rate testing and stomata response while more mature, harvested plants have been used for postharvest quality testing. Further research should focus on exploring and understanding the correlation between live plants and harvested plants at different maturity stages as it relates to photosynthetic rate, stomata response and postharvest quality. Another focus for future research should include using many LED lights across the spectrum, determining stomata opening aperture, light compensation points data, to more optimally determine which wavelengths are favorable for the extension of shelf life quality of various plant species. We did not find data specifically comparing narrow bandwidth green LED light and fluorescent light for postharvest storage of various plant species. Other studies might be difficult to interpret, experiments are usually performed using broadband spectrum that are not exclusively emitting green light. Future research should address this gap and further explore the effects of narrowband green LED light (500 – 560 nm) on the postharvest quality of plants while comparing it with other conventional lighting. More wavelengths in the green spectrum and plant species need to be included to provide a better understanding of the effects of narrow-bandwidth LEDs on plants' shelf life and visual quality.

In addition, the different light treatments in this experiment were done under room temperature storage. The shelf life quality of both spinach and kale deteriorated within 4 to 5 days. It would be beneficial to test the effects of green LEDs on the long-term storage of leafy vegetables under cool temperature storage (4 ℃).

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7. Appendix A – raw data

The figures below (Figure A1 – A6) are light compensation point data presenting photosynthetic rate (µmol m^{-2} s^{-1} of CO_2) and irradiance (µmol m^{-2} s^{-1}), showing the photosynthetic response of 14 different LED wavelengths (405, 417, 430, 449, 470, 501, 519, 530, 560, 575, 595, 624, 633 and 661 nm) under four irradiances (50, 100 and 150 µmol m^{-2} s^{-1}) including the dark respiration data. The figures were split into different sections: blue spectrum, green and red spectrum.

Other figures (Figure A7 – A8) include the replicates of shelf life testing after a 4-day storage. The treatments include dark, fluorescent, 500 nm and 560 nm. The visual quality of the dark treatment is below marketability (scale 2) but was shown for reference.

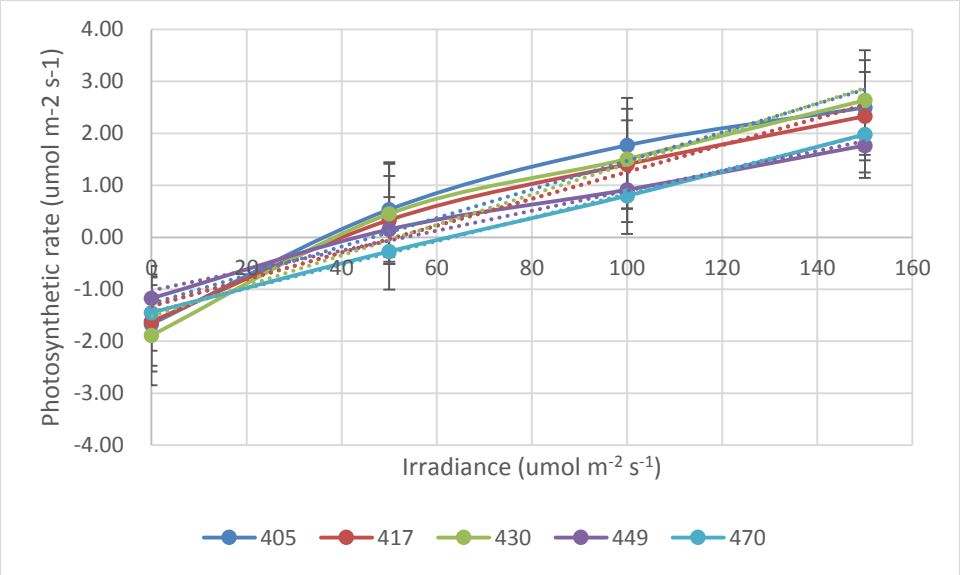

Figure A 1: PAR 'Melody'spinach (*Spinacia oleracea* L.). Results of average relative photosynthesis response of spinach. Light treatments of five wavelengths (405, 417, 430, 449 and 470 nm) at four irradiance levels 0, 50, 100 and 150 µmol m^{-2} s^{-1}.

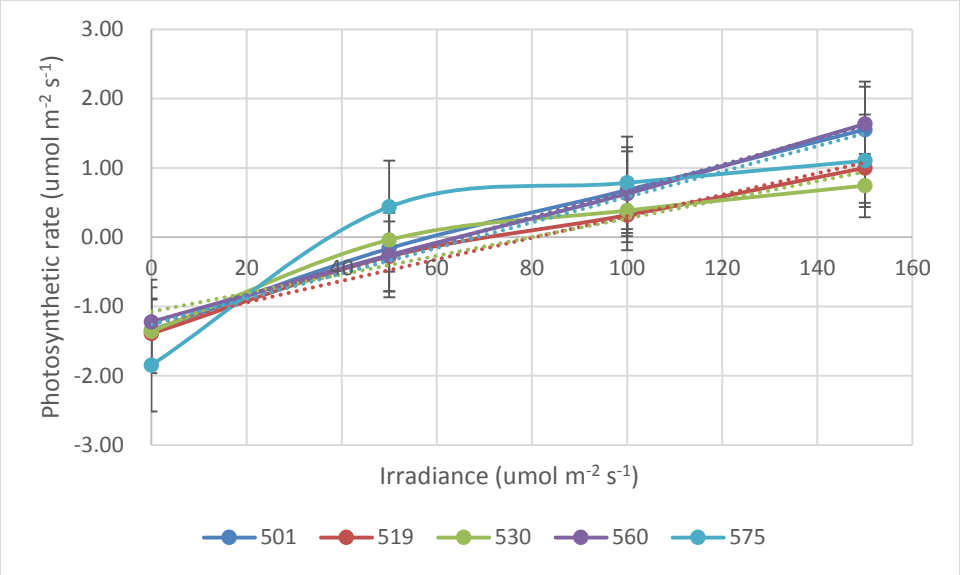

Figure A 2 : PAR 'Melody' spinach (*Spinacia oleracea* L.). Results of average relative photosynthesis response of spinach. Light treatments of five wavelengths (501, 519, 530, 560 and 575 nm) at four irradiance levels 0, 50, 100 and 150 µmol m^{-2} s^{-1}.

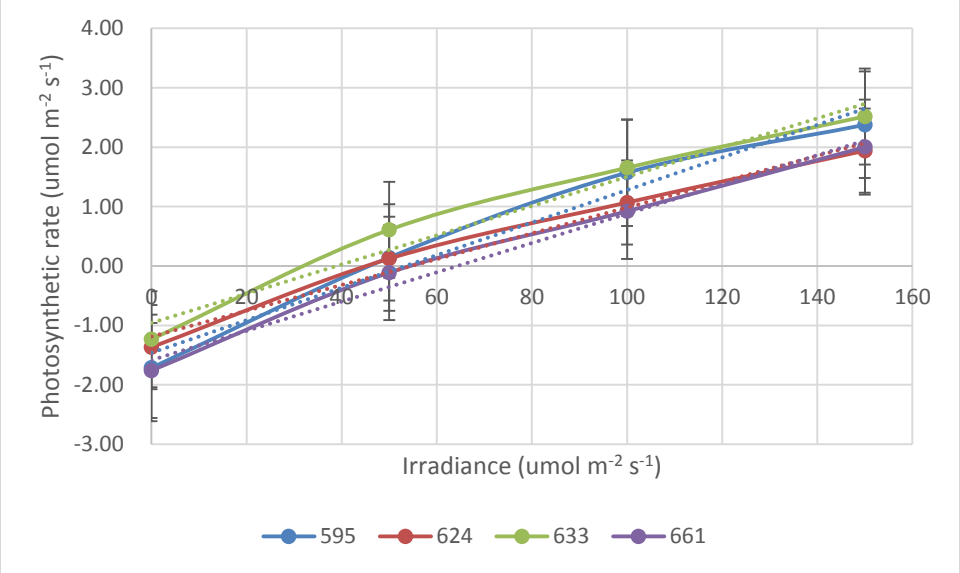

Figure A 3 : PAR 'Melody' spinach (*Spinacia oleracea* L.). Results of average relative photosynthesis response of spinach. Light treatments of four wavelengths (595, 624, 633 and 661 nm) at four irradiance levels 0, 50, 100 and 150 µmol m^{-2} s^{-1}.

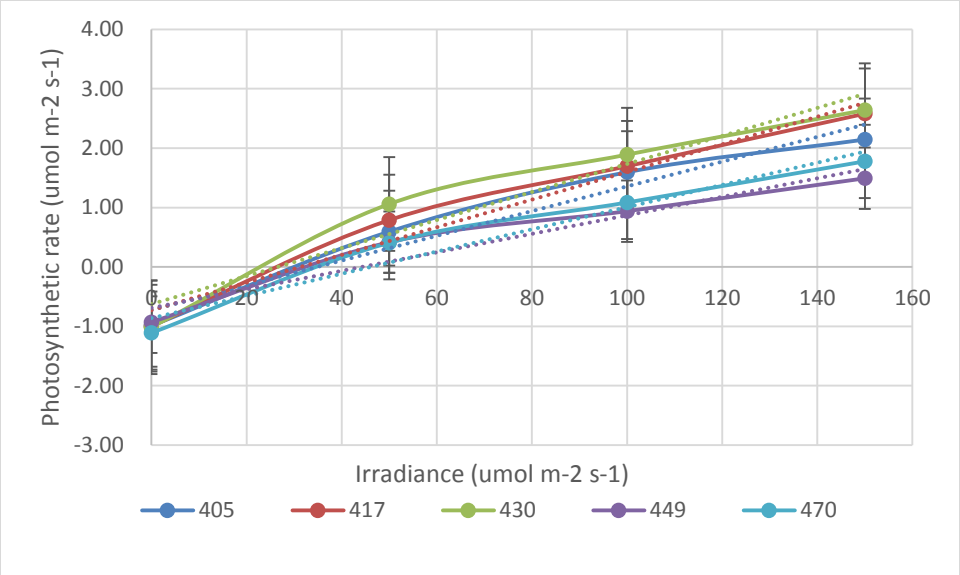

Figure A 4 : PAR 'Winterbor' kale (*Brassica oleracea* L.). Results of average relative photosynthesis response of kale. Light treatments of five wavelengths (405, 417, 430, 449 and 470 nm) at four irradiance levels 0, 50, 100 and 150 µmol m^{-2} s^{-1}.

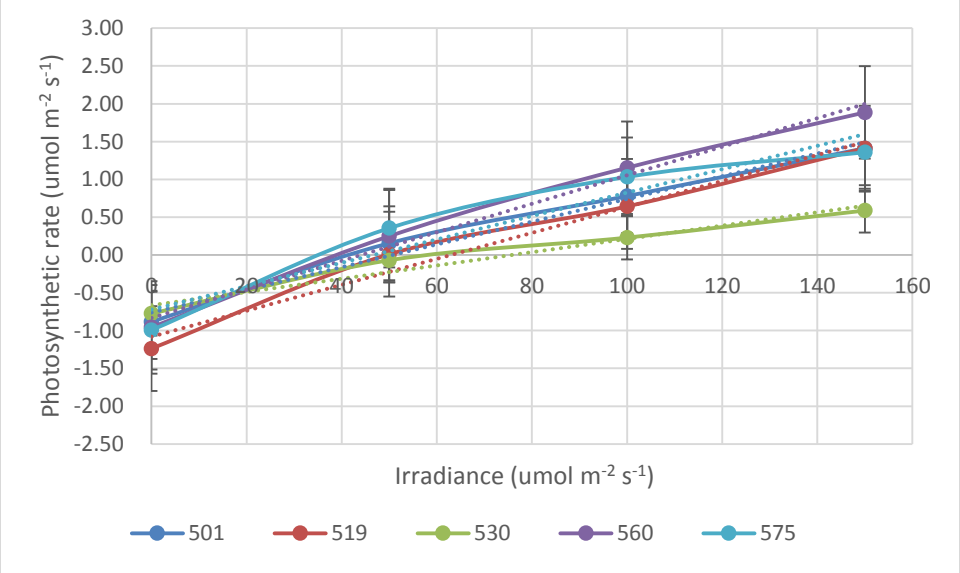

Figure A 5 : PAR 'Winterbor' kale (*Brassica oleracea* L.). Results of average relative photosynthesis response of kale. Light treatments of 5 wavelengths (501, 519,530, 560 and 575 nm) at four irradiance levels 0, 50, 100 and 150 µmol m^{-2} s^{-1}.

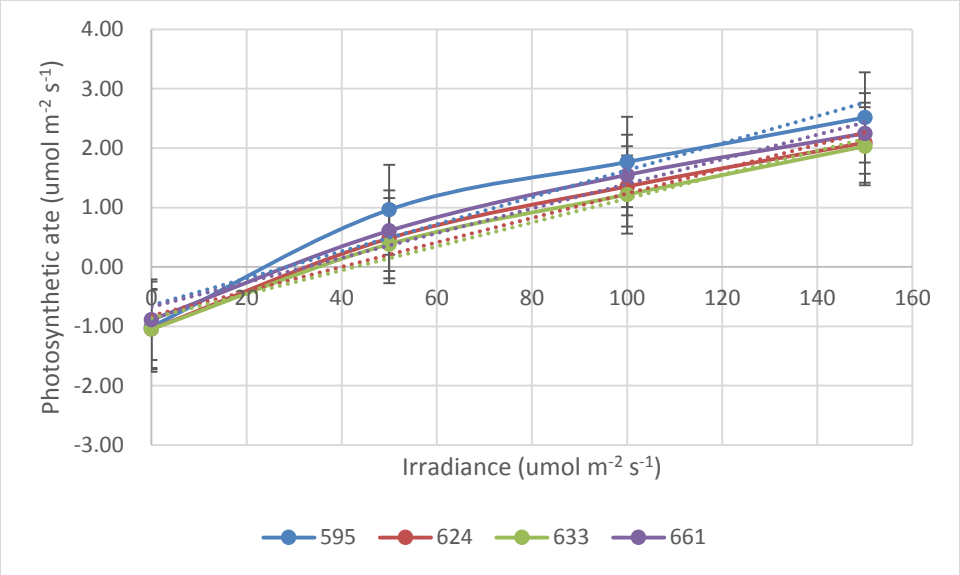

Figure A 6 : PAR 'Winterbor' kale (*Brassica oleracea* L.). Results of average relative photosynthesis response of kale. Light treatments of 4 wavelengths (595, 624, 633 and 661 nm) at four irradiance levels 0, 50, 100 and 150 µmol m^{-2} s^{-1}.

Figure A 7: The shelf life testing results after 4 days of storage: dark, fluorescent, 500 nm and 560 nm.

Figure A 8: The shelf life testing results after 4 days of storage: dark, fluorescent, 500 nm and 560 nm.