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The Effects of UV Radiation on the Content of Phenolic acid and Flavonoid, Stomatal Conductance and Taste in Red Lettuce 'Lollo Rosso'

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Abstract

The use of supplementary light to improve the quality of red lettuce is common practice in today's greenhouse production. Fluorescent lamps that emit Ultraviolet (UV) radiation have been shown to increase the synthesis of phenolic acids and flavonoids as well to control stomata movements in red lettuce. In the work presented here, I ran experiments which showed that the use of UV treatments lead to higher concentrations of these health-promoting compounds. The objective was to evaluate the effects of UV radiation on the synthesis of phenolic acids and flavonoids, as well as the stomatal conductance in red lettuce 'Lollo Rosso'. The plants were cultivated for 30 days under irradiation from HPS lights. They were then subjected to 7 days of UV treatment: Control (PAR), UV-A (PAR+UV-A) or UV-A+B (PAR+UV-A/UV-B). After the treatment, the stomatal conductance was measured and the chemical composition of the leaves was determined using high-performance liquid chromatography (HPLC). Subsequently, a sensory analysis was conducted in order to determine whether the UV treatment lead to a bitter taste in the red lettuce. The HPLC analyses showed that the PAR+UV-A/UV-B treatment gave the highest content of phenolic acids and flavonoids and also produced the most bitter lettuce. The plants under the PAR treatment had the lowest levels of phenolic acid and flavonoids and were considered to be the best tasting. Quercetin was the main flavonoid compound in the leaves, while chicoric acid and chlorogenic acid were the most prevalent phenolic acids. Overall, the mature leaves had higher levels of these compounds than the younger leaves. Furthermore, there was a statistically significant difference in stomatal conductance between the three treatments. The results indicate that the PAR+UV-A/UV-B treatment in greenhouses may induce higher concentrations of phenolic acids and flavonoids, with no detriment to stomatal conductance in red lettuce. Unfortunately the same treatment also leads to an undesirable bitter taste.

Keywords: red lettuce, UV radiation, greenhouses production, phenolic acid, flavonoids, stomatal conductance, taste.

Contents

List of abbreviation

CHI	Chalcone isomerase
CHS	Chalcone synthase
DAD	Diode array detector
FR	far-red
HPLC	High performance liquid chromatography
HPS lamps	High-pressure sodium discharge lamps
HIR	High-irradiance response
hTAS2R14	Human bitter taste receptor 14
hTAS2R39	Human bitter taste receptor 39
HY5	ELONGATED HYPOCOTYL5
Nm	Nanometer
NO	Nitric oxide
PAR	Photosynthetically active radiation
Phys	Phytochromes
R	red
ROS	Reactive oxygen species
SKP	Center for Plant Research in Controlled environment
TRC	Taste receptor cell
T2R	Type 2 receptor
UV	Ultraviolet rays
UVR8	UV RESISTANCE LOCUS8

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1. Introduction

Vascular plants have many different types of polyphenolic compounds and occur they naturally in plants as result of plants secondary metabolism. These compounds are normally attached to sugars and arise biogenetically from two metabolic processes known as shikimic acid and malonic acid pathways. About 8000 different types of polyphenols are actually known and the flavonoids are the most abundant polyphenolic compound (Bravo, 1998, Ross and Kasum, 2002). Flavonoids are responsible for the beautiful pigmentation in plants, fruits, vegetables (Schijlen et al., 2004). Furthermore they have several functions in plant systems such as reproduction, physiological, seed dispersal. In addition flavonoids also play a role in the photosynthetic mechanism and provide environmental stress protection from ultraviolet rays (UV) acting as a light screen against damage (Yao et al., 2004).

UV affects phenolic acid and flavonoid synthesis and the stomata movements in many different ways. The synthesis of these compounds is highly related to UV (Hagen, 2006) and most phenolic compound and flavonoid synthesis increase under UV radiation (Winkel-Shirley, 2002, Tossi et al., 2011). The role of flavonoids in plant's protection against UV is supported in many studies (Winkel-Shirley, 2002, Ryan et al., 2001, Agati et al., 2013, Bieza and Lois, 2001). In the same way the process of stomata conductance is highly affected to UV exposure (Nogués et al., 1999, Giannini et al., 1996). Several studies observed that UV might lead to decreased stomatal conductance in field conditions (Jansen and Van Den Noort, 2000, Giannini et al., 1996) and under greenhouse conditions (Nogués et al., 1999).

The aim of this study was to investigate the phenolic acid, flavonoid synthesis, stomata conductance and the taste in Lollo Rosso treated with PAR, UV-A and UV-B radiation in greenhouse conditions. Phenolic acids and flavonoids accumulation as response to UV radiation varies between species and in different radiations. These compounds are suggested by epidemiological researches and their effects in vitro and in vivo, to have many health benefits and prevent humans sickness such as coronary heart disease and cancer (Patel, 2008,

Ross and Kasum, 2002). The health potential is mainly result of their free radical scavenging effect and the potential antioxidants (Patel, 2008, Yao et al., 2004, Ross and Kasum, 2002).

Stomata conductance is also suggested to respond differently under different UV radiation. Furthermore, UV radiation may lead to accumulation of compounds that have a bitter taste in red lettuce. Due to the nutrition merit, 'Lollo Rosso' is an important source of phenolic compounds and bioflavonoids. In addition red lettuce has a high potential economic value. These results may be useful to improve the quality and increase the synthesis of polyphenolic compounds in red lettuce. In northern latitudes during the winter the greenhouses production tends to have lower quality due to the reduced or absent natural-radiation. In general greenhouses have cladding that not transmits UV radiation. Even the greenhouses that transmit UV do not fix the problem since there is no UVB radiation during the winter. Further, the lights that are commonly employed in greenhouses have low or no UV radiation (Rodriguez et al., 2014).

1.1. Literature Review

UV is emitted from the sun and covers wavelengths in the range 100-400 nm. Biologically, this range is divided in three bands: UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (100–280 nm). UV-A radiation that covers the range (315-400nm) reaches the earth surface in large scale while UV-B radiation is partly absorbed in the atmosphere and only a small portion reaches the earth surface. UV-C radiation cannot penetrate the atmosphere (Madronich et al., 1998). The proportion of UV radiation that reaches the Earth's surface is inversely related to the amount that is absorbed by stratospheric ozone (Stapleton, 1992).

1.1.1. The use of light in greenhouse

In Norway the use of light supplement that transmit UV radiation in greenhouse is increasing. Norway is located at high latitude approximately 59° N to 71° N. Due to this geographic position supplement of UV lighting may be necessary (Moe et al., 2005). During the wintertime the production in greenhouse became possible due to use of supplement of lights. According to Moe et al. (2005) Norway have the most effective greenhouse production considering the area of cultivation and the food quality. Efforts to improve greenhouse food quality are the main objective in this research today.

HPS lamps are the most common lamp type used in greenhouses. This lamp has a spectrum from blue to red and but looks orange (Aphalo et al., 2012). These lamps have a lower blue light portion. The natural sunlight have approximately (18%) and the HPS lamps around (5%) of blue light. There is an increased on interest in research to use lamps that emits UV radiation such as fluorescents lamps in greenhouses. The most common fluorescents lamps used in greenhouse experiments are UVA-340 and UVB-313 (Aphalo et al., 2012). According to the fabricant (Q-LAB) UVA-340 lamps peaks a 340nm and are the best simulation to the sunlight being the best to compare with the outdoor investigations. The UVB-313 lamps emits short wavelengths because these lamps emits an unnatural short wavelengths may induce to errors in indoors investigations. UV fluorescents lamps are reported to have a role in the leaf shape and plant colour (Grimstad, 1982).

1.1.2. Light photoreceptors

Plants are able to sense visible light and UV radiation through different photoreceptors, which have been identified in plants. Plant responses to light quality are mediated by different photoreceptors: pytochromes for red (R) and far-red (FR), phototropin and cryptochrome, for blue light and UV-A, and UV RESISTANCE LOCUS8 (UVR-8) for UV-B (Wu et al., 2012). These photoreceptors have different, and sometimes overlapping, functions in plant systems.

Phytochromes (phys) are photoreceptors that absorb mainly light in the R, FR and blue light spectrum but also in the UV area. Different light quality and amount of light induces the different phytochromes' response. Phys play a role in the phototropism process, morphology and some plant photomorphogenic responses such as flowering, synthesis of anthocyanin, and the production of ethylene (Smith, 2000, Taiz, 2010). The synthesis of anthocyanin in some seedlings and in apple skin may is induced as a phytochromes response by high irradiance response (HIRs) (Taiz, 2010, Saure, 1990). The phytochromes may have a role in synthesis of anthocyanin in young cabbage (*Brassica oleracea* cv. *Red Acre*) in continuous exposure to far red irradiation (Mancinelli and Rabino, 1975). Further, phytochromes may also contribute to stomata opening as a modulator of the blue response (Shimazaki et al., 2007).

The phototropins (phot1 and phot2) are blue light receptors and are involved in the photosynthetic process. They play a role in blue-light-induced stomata opening, mediate phototropism, leaf expansion, plant growth and chloroplast movement in response to light intensity (Christie, 2007, Taiz and Zeiger, 2010). Cryptochromes (CRY1 and CRY2) are a flavoproteins that mediate the blue light receptors (Ahmad et al., 1998). They are important in

photomorphogenic responses such as phototropism and synthesis of anthocyanin (Ahmad et al., 1998, Möglich et al., 2010, Christie, 2007).

Plants respond to UV-B radiation via the UVR8 photoreceptor. This response occurs when plants are exposed to UV radiation of short wavelengths or are damaged by UV radiation. UVR-8 are proteins that accumulate in plants and regulate many genes involved in UV-B responses (Christie, 2007, Wu et al., 2012, Jenkins, 2009, Jenkins, 2014, Cen and Bornman, 1990). The UVR-8 photoreceptor regulates genes related to the concentration of secondary compounds such as phenolic acid and flavonoids. These compounds are able to reduce the UV-B penetration into the leaf and act as antioxidants protecting the plants (Julkunen-Tiitto et al., 2005, Jenkins, 2014), increased leaf thickness (Teramura and Sullivan, 1994, Liu et al., 1995) and reduction in height and leaf area in the growth of plants (Deckmyn and Impens, 1998, Jansen et al., 1998)

However, UV-B radiation in plants is not only related to damage but may also be considered as a kind of signal for the photomorphogenic mechanism in plants (Julkunen-Tiitto et al., 1996, Wargent et al., 2009). In fact, UV-B radiation regulates hormones related to the photomorphogenic mechanism (Wargent et al., 2009), morphologic changes such as increased branching (Sullivan et al., 1994, Newsham et al., 1999)

1.2. Stomatal Response to UV Radiation

Stomata opening allows gaseous exchange (CO_2 and H_2O) into and out of leaves. This process occurs naturally in different environmental conditions and is regulated by air humidity, air movements CO_2, temperature, water supply and radiation. The environmental conditions are sensed by the guard cells and these cells and regulated by plants hormones and second messengers to induce signals and control stomatal movements (Taiz, 2010). The most important plant phytohormone involved in stomatal closure is abscisic acid (ABA) (Mishra et al., 2006). Also other hormones like ethylene, cytokinin and auxin are involved in stomatal opening and closing (Watkins et al., 2014, Tanaka et al., 2008).

The process of stomata movements and plant transpiration is highly affected to by light including blue and red light (Zeiger, 1983). Several studies observed that UV radiation might lead to decreased stomatal conductance in field conditions an under greenhouse (Jansen and Van Den Noort, 2000, Giannini et al., 1996, Nogués et al., 1999). UV radiation has also been

reported to stimulate both stomata opening and closure (He et al., 2005). It seems to depend on which wavelength plants are exposed to (Wargent et al., 2009). In addition it also depends on species**.** For instance, UV-B is reported to decrease stomata conductance in pea under greenhouse conditions (Nogués et al., 1999). While, UV-B induced increase stomata conductance in *Ericaceae* was observed by Musil and Wand (1993). The reason for that may be the diverse morphology of the guard cells. Guard cells have different wall structures some portions are substantially thickened than others. This difference in the wall structures plays an important role in the opening and closing stomata (Taiz, 2010). Moreover, stomatal conductance is affected by the intensity of environmental conditions such as light intensity, water supply or plants hormones (Jansen and Van Den Noort, 2000)

Different photoreceptors are involved in UV induced and changes stomatal conductance (Taiz, 2010). The UV-A radiation is probably sensed by the photoreceptors, phot1 and phot2 (Chen et al., 2012). However, UV-B induced changes in stomatal conductance and the aperture is regulated vie the (UVR8) receptor. UVR8 mediates stomatal closure that is regulated by nitric oxide (NO), NO controls several processes in plants and plays a role in stomatal closure (Tossi et al., 2014). According to Tossi et al. (2014) UVR8 signals involve CONSTIUTIVELY PHOTOMORPHOGENIC1, the ELONGATED HYPOCOPYL5 (HY5) transcription factor, and a closely related HY5 HOMOLOG.

In a study done with Arabidopsis as a model, the authors Tossi et al. (2014) investigated the UVR8 pathway and its interaction with nitric oxide (NO) and hydrogen peroxide (H_2O_2). According to the authors UV-B radiation mediated by UVR8 increases of both NO and H_2O_2 in guard cells allowing stomata closure. The involvement of ROS in UV induced movements was also reported by He et al. (2005) but UVR8 was unknown at that time. The Tossi et al. (2014) model explains how UVR8 induce stomata closure (see Figure 1 the model).

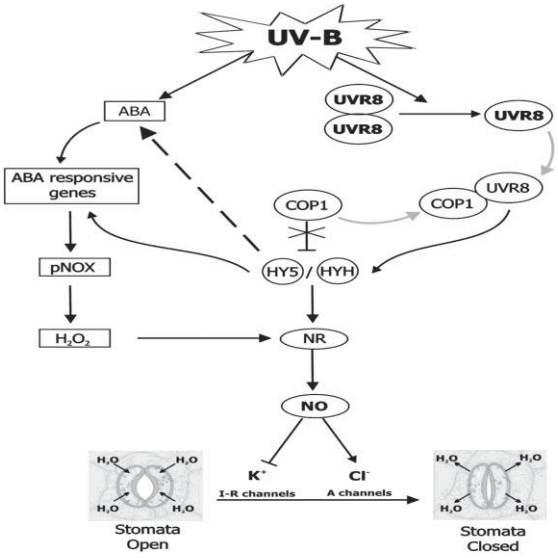

Figure 1. Demonstrated the UVR8 pathway in UV-B-induced stomatal closure. Black arrows indicate induction. Black bars indicate negative regulation. Gray arrows indicate protein interaction and rearrangement and dashed line indicates hypothetical cell response. I-R, Inward-rectifying K^+ channel; A, anion channel. Adapted from Tossi et al. (2014)

1.3. Phenolic Acids and Flavonoids - Synthesis Under UV Radiation

According to Bravo (1998) and Ross and Kasum (2002) about 8000 different types of polyphenols is actually known. Phenolic compounds are divides in different groups the phenolics acids, flavonoids, stilbenes, coumarins and tannins and generally have one or more rings with one or more hydroxyl groups (Liu, 2004). Phenolic acids have a basic phenylpropanoid carbon skeleton (see Figure 2).

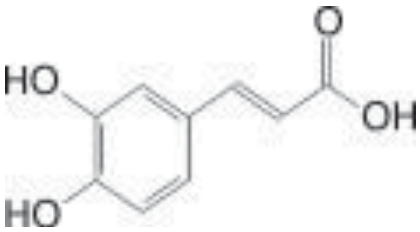

Figure 2. Chemical structure of a simples phenolic compound. Adapted from de Souza and Spinelli (2009)

The flavonoids are the group with a large number of phenolic compounds. The literature diverges on the number of flavonoids that have been identified, between 4000 (Dugo et al., 2005, Iwashina, 2000) and 6000 (Schijlen et al., 2004), unique flavonoids are known from vegetal sources and these number tend to increase. These compounds vary in chemical structure but in general all have the same basic structure – a C_6-C_3-C_6 carbon skeleton, consist

of two aromatic rings interconnected by a three-carbon bridge commonly cyclised with oxygen (see Figure 3) (Hagen, 2006, Yao et al., 2004, Pekkarinen et al., 1999, Middleton et al., 2000). These three phenolic rings are referred to as A, B and C rings, and depending on the C-rings structure, nature and number, they are classified in to different groups and different chemical proprieties (Yao et al., 2004, Hagen, 2006).

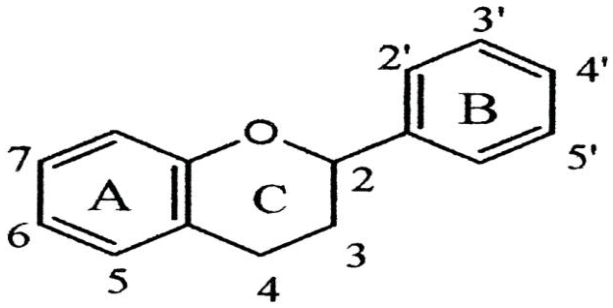

Figure 3. Basic flavonoid carbon skeleton. Flavonoids contain 15 carbons in the basic molecular skeleton provided by two aromatic rings and one 3-carbon bridge. Numbers shows the position of carbon on the flavonoid rind system. Adapted from Taiz (2010) and (Liu, 2004)

The main groups comprise flavanones, flavones, isoflavonoids, flavonols, anthocyanidin; (Yao et al., 2004, Hagen, 2006). The chemical structure of flavonoids related to their light absorption in different wavelengths. They have different peaks within two main absorption bands, the main absorption bands are Band I (320-385 nm) that corresponds to the B ring absorption and Band II (250-285) which corresponds to the A ring absorption Yao et al. (2004).

Flavanones have a great peak in Band II and contribute to the bitter taste and flavor of citrus neohesperidose flavanones, such as naringin found in grapefruit (Yao et al., 2004). Flavonols absorb mainly in both bands (I and II). They are the most common flavonoid in food and have a pale yellow colour. Flavonols are abundant in onions, cherries and apples and have the main groups kaempferol, quercetin and myricetin. Flavones also absorb in both bands (I and II), are most common in herbs, vegetables and flowers. They may have a bitter taste, luteolin are examples of flavones (Yao et al., 2004).

Anthocyanidin glycosides are the red, blue and violet pigments in plants they absorb in the visible light spectrum particularly green light and to lesser extent blue light (Edreva, 2005), and give flowers, fruits and leaves colours. Cyanidin glycosides are the most common type of anthocyanidin (Rodriguez et al., 2014, Yao et al., 2004). Anthocyanidin peaks in the two bands (I and II) depending on the attached chemical group (Yao et al., 2004).

The major phenolics acids in red lettuce are chicoric acid (dicaffeoyltartaric acid), chlorogenic acid (5-O-caffeoylquinic acid) and caffeic acid (caffeoylmalic acid) (Llorach et al., 2008, Romani et al., 2002). Chlorogenic acids are reported to act as antioxidants, act as plant protection against pathogens (Niggeweg et al., 2004, Tamagnone et al., 1998) and additionally protect plants against UV radiation (Tegelberg et al., 2004). According to Oh et al. (2009) chicory acid and caffeic acid accumulate under high light stress and may show the same pattern as chlorogenic acid. The synthesis of chicoric acid is still not well investigated.

Research on phenolic compounds and flavonoids has increased over the last few years, primarily their multiple functionalities in plant systems (Saito et al., 2013), in addition to the their role in protection against UV (Winkel-Shirley, 2002, Agati et al., 2013, Degl'Innoocenti et al., 2008). Accumulation of flavonoids offer are plants protection against these factors (Degl'Innoocenti et al., 2008, Winkel-Shirley, 2002). UV radiation has been identified by Ryan et al. (2001) as inducing phenolic compounds and flavonoid synthesis with higher hydroxylation levels. This study was done with *Arabidopsis* and showed that at higher levels of hydroxylation, UV radiation affects their antioxidant capacity, suggesting that flavonols may play a role in UV stress response (Winkel-Shirley, 2002). Similar study on plants response to UV was done by Brazaitytė et al. (2015) this study evaluated the effects of UV-A radiation in microgreens according to the authors, UV-A treatment, depending on the species, can increase synthesis of antioxidants such as anthocyanins

1.4. Red Lettuce 'Lollo Rosso' and the Taste

The red lettuce 'Lollo Rosso' belongs to the *Asteraceae* family, and is one of the most popular lettuce types consumed wide word. 'Lollo Rosso' is considered a "healthier" food and consumption of it is increasing. The perception it as a healthier food is due to the red pigmentation in the plant tissues, in response to the amount of different type of phenolic compound and flavonoid (Llorach et al., 2008). Lollo Rosso has a nutritional value related to the phenolic acid and flavonoid content and their antioxidant and free-radical-scavenging properties (Ferreres et al., 1997, Crozier et al., 1997).

Although red lettuce has important phytochemicals and biological proprieties and functions such anti-inflammatory, analgesic, anti-tumor in addition and source of iron, potassium and fibre (Chadwick et al., 2016), they can have a bitter taste. All consumers do not appreciate the bitter taste in red lettuce. Bitter taste in lettuce may be related to the chemical compounds

such as sesquiterpene lactones (Chadwick et al., 2016, Price et al., 1990) and some others types of flavonoid. In fact, many flavonoids are reported to have a bitter taste for instance quercetin, naringin, epicatechin, catechin, isoflavone glucosides (Chadwick et al., 2016, Drewnowski and Gomez-Carneros, 2000).

In humans, bitter tastes are sensed by bitter receptors on the tongue. The bitter molecules bind to G-protein-coupled receptors located in the membrane of the taste receptor cell (TRC) in the taste buds (Sessa et al., 2000). The bitter molecules bind to type 2 receptors (T2Rs), of which there are 25 involved in bitter taste perception (Chadwick et al., 2016). The human bitter taste receptors hTAS2R14 and hTAS2R39 are known to be activated by a large number of chemical compounds that include isoflavonoids and flavonoids (Roland et al., 2011, Roland et al., 2013). Roland et al. (2013) elucidated that the two human bitter receptors hTAS2R14 and hTAS2R39 may be responsible for detecting the bitter taste of flavonols. Based on the flavonols' chemical group and their structure, the authors suggest that flavonols have chemical characteristics typical of many bitter compounds and are mostly detected by the receptor hTAS2R39.

The main types of phenolic compound and flavonoid content in lettuce red lettuce are chicoric acid (dicaffeoyltartaric acid), chlorogenic acid (5-O-caffeoylquinic acid), caffeoylmalic acid, quercetin, quercetin derivates, cyanidin 3-malonylglucoside, luteolin-7-O- glucuronide, luteolin -7-O- glucoside, luteolin 7-O- rutinoside (Ferreres et al., 1997, Crozier et al., 1997, Behn et al., 2011, Caldwell, 2003, Llorach et al., 2008) (see Figure 4).

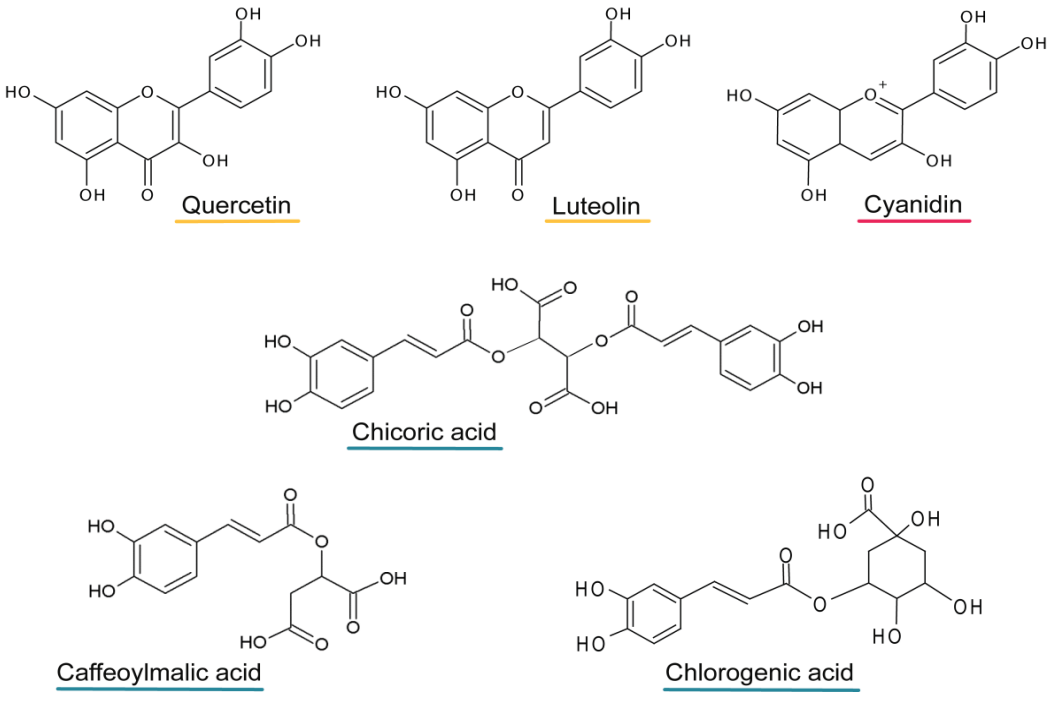

Figure 4. The main flavonoid aglycones and caffeic acid derivatives in red leaf lettuce: quercetin, luteolin, cyanidin, chicoric acid (di-*O*-caffeoyltartaric acid), chlorogenic acid (5-*O*-caffeoylquinic acid), *O*-caffeoylmalic acid. Compound names are supported by colored lines, which are pointing out the different chemical classes. Adapted from Becker (2014)

The main nutritional properties of the phenolic acids and flavonoids identified in red lettuce 'Lollo Rosso' are:

- Chicoric acid - in human health this helps prevent diseases such as cancer and diabetes (Lee and Scagel, 2013) and may be a important tool in the treatment of obesity (Xiao et al., 2013). Furthermore, this compound is reported to have antiviral properties. According to Queffélec et al. (2008) chicoric acid is a useful agent in HIV treatment.
- Chlorogenic acid is the phenolic acid most available to humans since they are absorbed directly by the small intestine. They are powerful antioxidants and suggest to may prevent carcinogenesis an atherosclerosis (Niggeweg et al., 2004)
- Quercetin glycosides are reported to be potent antioxidants associated with a reduction of coronary heart disease and stroke (Ross and Kasum, 2002). In addition they are thought to may help prevent neurodegenerative diseases such as Alzheimer's and Parkinson's due to its antioxidant and free-radical scavenging properties (Jan et al., 2010).
- Cyanidin's are reported to be potent inhibitors of lipid peroxidation and are powerful antioxidants. They are related to some antioxidants specific to prevention of diseases such as cardiovascular disease and cancer and protect against urinar infection. Furthemore, they are beneficial to ocular and dermal health (Zafra-Stone et al., 2007).

1.5. Objective of the Study

Lettuce cultivated in greenhouses during the winter needs to be supplemented with extra photosynthetically active light. During the winter in northern latitudes there is low or no UVB radiation to induce the synthesis of phenolic acids and flavonoids which may be responsible for the color and taste of red lettuce (Becker et al., 2014b). These compounds (phenolic acids and flavonoids) are also reported to promote human health, being valuable to the food industry. Furthermore, since UV radiation affect plant stomatal conductance it may be used as a tool to control transpiration in greenhouse production (Giannini et al., 1996, Jansen and Van Den Noort, 2000).

1. Does UV-A and UV-B affect synthesis of phenolic acids and flavonoids in red lettuce cultivated in greenhouses?
2. Does UV radiation synthesize phenolic acids and flavonoids that may lead to a stronger taste in red lettuce?
3. Is the stomatal conductance affected by the treatments applied?

2. Methods

2.1. Plant Material and Experimental Facilities

The experiments were performed at the Center for Plant Research in Controlled Environment (SKP) at Norwegian University of Life Sciences (NMBU) during September 2015 to December 2015.

Seeds of *Lactuca sativa* 'Carmoli' RZ 85-85, Lollo Rosso NORGRO As Pb 4144, 2307 Hamar were sown direct in 12 cm pots filled with Sphagnum peat pH 5.0–6.0, salinity ca. 1,5–2.5, Degernes Torvstrøfabbrikk AS, Degernes, Norway. Further, the plants were watered daily using the standard system feed use in the SKP, a nutrient solution with an electric conductivity of 1.5 $mS\text{-}^{1}$ and pH of 5.5. The nutrient solution was mixture of Red Superba and Calcinit (Yara, Norge AS, Oslo).

2.2. Growth Chamber Conditions

The plants were grown in a greenhouse compartment with acrylic walls and a glass roof. Further, they were covered with a polycarbonate (4mm) in order to block any exterior UV radiation penetrating the glass roof (see Figure 5).

Figure 5. Red lettuce 'Lollo Rosso' growing in the growth chamber under HPS light

The air temperature in the greenhouse compartment was constant at 21° (±) 2° the relative air humidity (RH) was 70 % and the level of CO_2 (400 ppm). The climate data was controlled and collected using a greenhouse computer system PRIVA (Priva, De lier, The Netherlands).

The greenhouse compartment was illuminated with high-pressure sodium lamps (HPS) (Philips Master Sont-T PIA plus 400w E E40, Belgium) at a photosynthetically active

radiation (PAR) of 100-µmol m^{-2} s^{-1}. The light intensity was measured using a Li-Cor Quantum sensor with a Li-Cor Model L1- 250 (Li–Cor Inc., Lincoln, NE USA) light meter. The plants were given a photoperiod of 16 h light and 8 h dark period. After 5 weeks of pre-cultivation, when the plants had developed 8-10 leaves they were transferred to a closed greenhouse compartment with no natural light and exposed to different UV exposure (see Figure 6).

Figure 6. Red lettuce 'Lollo Rosso' grown in the growth chamber under HPS light for five weeks

The seeds were sowed in two dates: The first sowing was on 8th September and the second 14th October. The plants were transferred to the UV exposure on the 12th October and 18th November.

Experimental set –up:

Figure 7. Red lettuce 'Lollo Rosso' under the UV treatment the plants were exposed during 7 days.

The UV treatments were done according to the table below (see Table 1). The PAR was provided by high-pressure sodium (HPS) lamp (Philips Master Sont-T PIA plus 400w E E40 Made in Belgium) at photosynthetic photon flux density of 170µmol m^{-2} S^{-1}. Measured using Li-Cor Model L1- 250 Quantum sensor (Li–Cor Inc., Lincoln, NE USA). The photoperiod was similar as for the pre-cultivation and the UV-A and UV-A+UV-B treatments were given during the photoperiod (16 hr light and 8 hr darkness).

The UV-A treatment was provided by fluorescents tubes Q- Panel 340 CO. USA – Made in Canada. A polyester film was used to block any levels of ultraviolet-B (UV-B). The treatment UVA+UVB treatment was provided with the same tubes but without the polyester film.

A UV sensor was used to measure the UV-A and UV-B radiation Skye SKU 430 Sensor connected to a Skye SpectroSense2 Meter, (Skye Instruments Ltd, Llandrindod Wells, Powys, UK). The UV sensor was calibrated with an Optronic OL756 Spectroradiometer (Optronic Laboratories, Inc., Florida USA).

Table 1

Experimental set up of the pre-cultivation treatment in the closed greenhouse room. Photosynthetic active radiation in µmol m^{-2} s^{-1} at lettuce level and UV radiation provide from UVA fluorescents tubes

Measurements	T1: Control PAR	T2: PAR+UV-A	T3: PAR+UV-A +UV-B
PAR (µmol $m^{-2}s^{-1}$)	170	170	170
UV-B (W/m^2)	0.0023	0.0008	0.1543
UV-A (W/m^2)	0.3008	2.7707	3.6453
Filter	-	Polyester film	No film

The spectral wavelengths of the different light sources were measured with the Optronic OL756 Spectroradiometer (Optronic Laboratories, Inc., Florida USA) (see Figure 8).

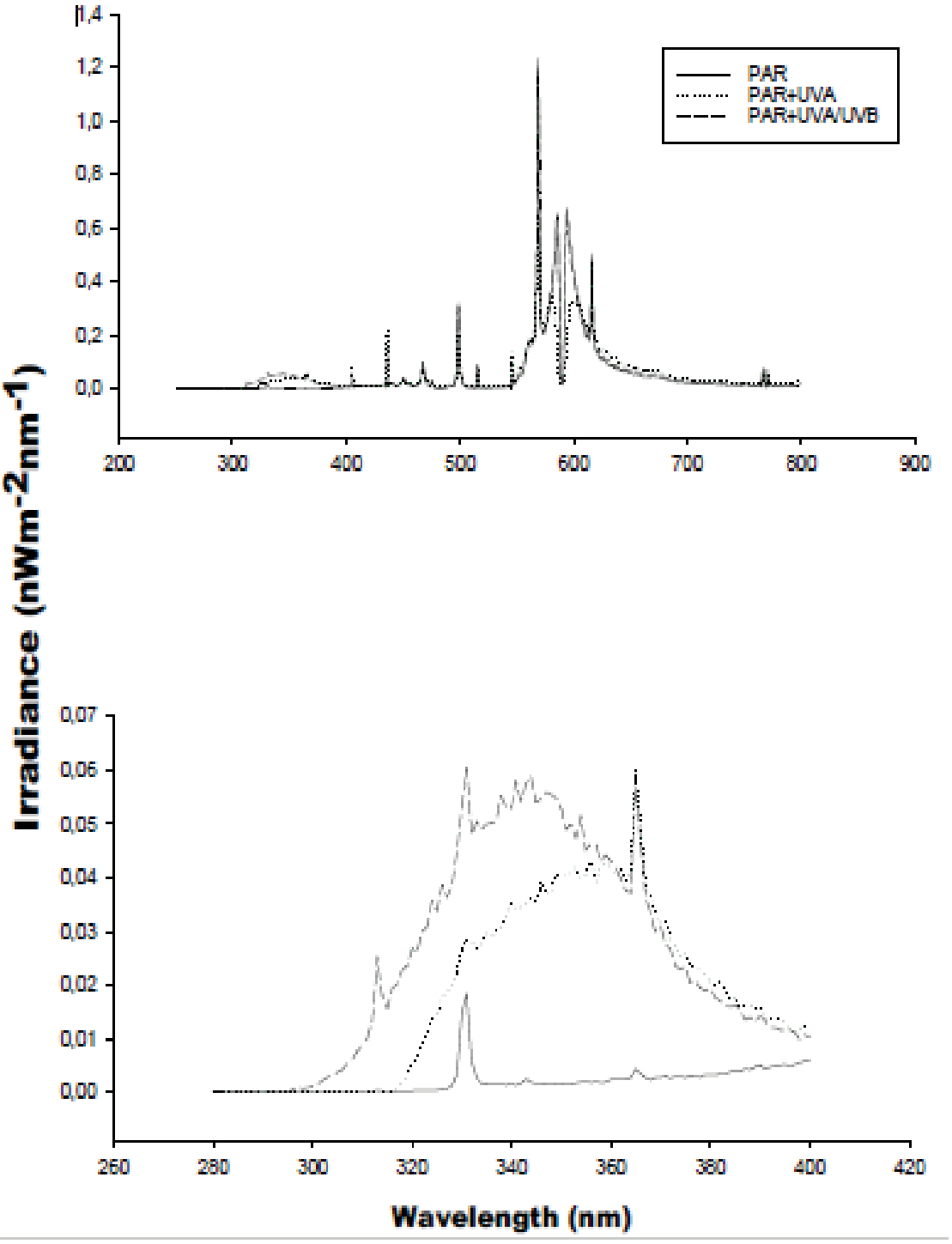

Figure 8. Irradiance spectra in growth chambers of the PAR (HPS lamps only), PAR+UVA (HPS lamps + UVA340 tubes screened with polyester) and PAR+UVA+UVB (HPS lamps + unscreened UVA 34 tubes)

2.3. Data Collection

2.3.1. Stomata conductance measurements

Stomata conductance measurements were performed in the growth room by the use of porometer (AP4 Delta –T Devices Ltd., Cambridge, UK). The first measurement was made on 19th October; eight days after the plants were exposed to the three different treatments. These measurements were taken in the light period in the mid-morning between 10 to 12 hours, two hours after the light was turned on.

The measurements were taken from seven young non-expanded around (5 to 10 cm) (centimetres) and seven mature fully expanded leaves from each treatment. These measurements were duplicated in each leaf and were taken on both upper (adaxial) and lower (abaxial) sides since lettuce has stomata on both sides of the leaves.

2.3.2. Samples for chemical analyses

In order to assess flavonoid synthesis over the plant leaves of different growth stage were sampled. The same leaves that were used for the stomatal conductance measurements were harvested for the chemical analyses. The samples consisted of young non-expanded leaves and mature fully expanded leaves from each plant. Twenty-one (21) samples from young non-expanded and twenty-one (21) samples mature fully expanded leaves were harvested for the chemical analyses high-performance liquid chromatography (HPLC). The samples were immediately frozen in liquid nitrogen (N_2) put in plastic tubes (10 ml) and stored at -80°C. Further, the samples were freeze-dried using a freeze dryer machine (Heto Holten A/S, Gydevang 17-19, DK-3450 Allerød, Denmark) (Islam et al., 2014).

2.3.3. Flavonoid extraction and HPLC analyses

This experiment was conducted at Department of Ecology and Natural Resource Management (INA). Flavonoids were determined HPLC (Agilent, Series 1100, Germany), consisting of a binary pump (G1312A), a thermostat autosampler (G1329A), a thermostat column oven (G1316A) and a diode array detector (DAD) (G1315B). The compounds were separated using a Thermo Scientific (ODS Hypersil 50 x 4,6 mm) column**.** The auto injection volume was 20µl, and all runs was performed at 30°.

2.3.4. Pigmentation extraction of Lettuce

In the first step, freeze dried leaves were grinded with a ceramic mortar and a pestle, using approximately 20 mg for the pigmentation extraction. The ceramic mortar and the pestle were cleaned each time before the next weighing. The dried extract was put into a vial. The second step was to add 600µl of MeOH (methanol) to the dried residue extract and the homogenized for 30s seconds. The vial was left for 15 minutes in an ice bath. The mixture was then centrifuged at 12 000 rpm for 3 min at high speed using an Eppendorf centrifuge AG 22331 (Made in Germany). Then the liquid supernatant was put into a marked reagent vial with a pipette. This second process was repeated and the supernatants were collected in the same reagent vial with a pipette.

The third step, the MeOH was evaporated from the supernatant with a vacuum centrifuge using an Eppendorf concentrator plus AG 22331 (Made in Germany). The dried extract was then stored in the freezer (-20°) until the analysis.

The fourth step, the dried extracts were dissolved in 200µl MeOH and 200µl of H_2O. The extracts were left in the ultrasound bath for 5 min. Then the extract was transferred with a pipette to a new vial and centrifuged for 3 min at high speed. After the centrifugation the clear extract was transferred to an HPLC-vial a lid was and put on for the analysis. The identification of compounds was based on retention times and spectra of the peaks compared with retention times and UV spectra according to the literature (Julkunen-Tiitto et al., 1996).

2.3.5. Quantification of the lettuce compounds

The quantification and concentration of the compounds was calculated as peak area of the compound versus response factor. The diode array detector (DAD) used for quantification, was set to record chormatagramas at the following wavelengths 320nm, 360nm and 550nm. Cyanidin glycoside was quantified at 550 nm, quercetin glycoside at 360 nm, and chlorogenic acid at 320nm and chicoric acid at 320nm. The values were compared with the standards that were available according to the literature data (DuPont et al., 2000, Llorach et al., 2008) and correlated with the results. Standard substances of chicoric acid were purchased (Sigma chemical company). The results were reported as micrograms per 1 g of dry planter material (DW).

2.4. Sensory analysis

The sensorial analysis was organized in the same day as the stomata conductance measurements. There was conducted a blind taste test. This method was chosen because the lettuce colour may have an effect on participant's responses. The test person did not know which salad type they consumed. Participants were a random sample of workers and students at the university. Total of 33 people participated.

A random of the red lettuce was used for the taste test. Lettuces were harvested the same morning of the taste tests and were used within half an hour after harvest. Samples were labelled with arbitrary three-digit codes for each treatment 1 - PAR; 2 - PAR+ UVA; 3 - PAR+ UV-A/UV-B. There were offered lettuce samples from each treatment. Then were offered to the participants a glass of water between tastings, to clean their palate during the rest period. After the test, participants answered two questions.

The participants were asked the following questions:

1. Which salad do you like the most?
2. Which salad do you find most bitter taste?

2.5. Statistical Analyses

Differences among the means were compared using (ANOVA) GLM Analyse of variance. Tukey's HSD test for the population with equal variances was used the multiple comparisons at ($p< 0.05$). The data used in the stomata conductance analyses are presented as an average value of the measurements. The interaction effect (UV*Side) was determined using a One-Way Analysis of the mean.

The data used on HLPC analyse were required Log10 transformations for achieve the assumptions. The analysis sensorial data were analysed using a chi-Square test: X^2, ($p<0,05$).

Bar Graphs provide some graphical displays of the data. Data are quoted as mean ± standard error. Data analyses were done using Minitab statistical software version 17. All graphical presentations were performed by SigmaPlot version 13.

2.5.1. Photos

All photos were taken using a Samsung Galaxy 5, model SM-G900F Android version 5.0.

3. Result

PAR PAR+UVA PAR+UVA/UVB

Figure 9. Red lettuce 'Lollo Rosso' after 7 days under the UV treatments.

3.1. Stomatal conductance in mature and young leaves

3.1.1. Fully expanded mature leaves

The stomatal conductance of non-exposed fully expanded mature leaves showed a slightly higher conductance compared to the UV exposed plants. However, the result was not statistically significant (see Table 2). When comparing upper and lower leaf sides stomatal conductance was significantly higher on the upper side of the mature leaves in the three treatments. The mature leaves in the PAR treatment had the highest conductance compared to PAR+UV-A and PAR+UV-A/UV-B treatment (see Figure 10). There was a trend towards a lower conductance of leaves exposed to UV-A+UV-B also in mature leaves but the data was not statistical different.

Table 2

Output from the full model Analyse of variance (GLM) effects of the UV treatments on stomatal conductance of fully expanded mature leaves

Source	DF	F-value	P-value	Significance
UV	2	3.08	0.052	*
Side	1	50.67	<0.001	***
Experiment	1	15.13	<0.001	***
UV*side	2	0.64	0.53	NS
UV*Experiment	2	1.54	0.221	NS
side*Experiment	1	6.57	0.012	*
Error	74			

3.1.2. Young non-expanded leaves

In general, UV had no significant effect on stomatal conductance. However, the different leaf side (upper and lower) responded differently. When exposed to UV-A the upper side showed significantly higher conductance compared to the lower side. Further, when exposed to UV-B the upper side showed significantly lower conductance than the lower side but is not significant (see Table 3). However, the upper side exposed to UV-A+UV-B showed significantly lower conductance compared to leaves exposed to UV-A. In the control treatment no significant difference was found between the two sides (see Figure 10).

Table 3

Output from the full model Analyse of variance (GLM) effects of the UV treatments on stomatal conductance of stomatal conductance young non-expanded leaves

Source	DF	F-value	P-value	Significance
UV	2	1.48	0.233	NS
Side	1	1.46	0.230	NS
Experiment	1	10.60	0.002	**
UV*Side	2	5.32	0.007	**
UV*Experiment	2	0.31	0.731	NS
Side*Experiment	1	3.04	0.086	*
Error	74			

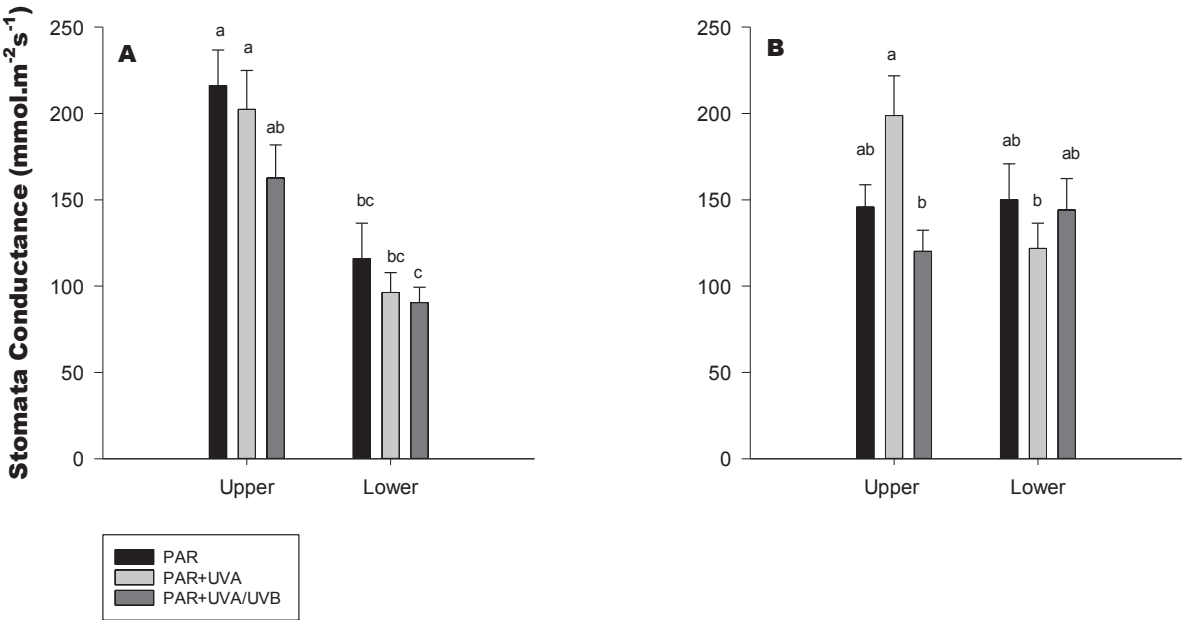

Figure 10. Stomatal conductance of lettuce 'Lollo Rosso' grown in UV deficient environment before transfer to UV exposure: Control (PAR) PAR, UV-A (PAR+UV-A), and UV-A+B (PAR+UV-A/UV-B), measured on the lower and upper leaf side of A) mature fully expanded leaves and B) for young non-expanded leaves and mature fully expanded leaves. The error bars show mean±standard error, n=7. Means within each development stage that do not share a similar letter are significantly different according to Tukey test, (p=0.05).

3.2. Flavonoids and Phenolic Compounds

The HPLC analyses show that quercetin glycosides were the main flavonoids compounds in 'Lollo Rosso'. Lettuce contains up to 16mg gDW-1 of quercetin, 5mg gDW-1 of quercetin glucosides, 13mg gDW-1 of chicoric acid (dicaffeoyltartaric acid), 10mg gDW-1 of chlorogenic acid *(5-O-caffeoylquinic acid*), 6mg gDW-1 cyanidin glycosides, 5mg DW-1 of quercetin derivate and three-type chlorogenic acid: the first 8.5, mg gDW-1 chlorogenic acid, chlorogenic acid 2.5mg aDW-1, chlorogenic acid 1.6mg gDW-1. The HPLC results show that the PAR+UV-A/UV-B treatment strongly induced the synthesis of the all compounds analysed. Generally, the mature leaves had higher concentrations of compounds than young leaves.

3.3. Flavonoids

3.3.1. Cyanidin glycosides

The HPLC chromatogram showed a substance with a spectrum with a peak at 520 nm. This spectrum corresponds to cyanidin glycosides. The levels of cyanidin glycosides were strongly induced by UV radiation (see Table 4). Lettuce under the PAR+UV-A/UV-B treatment had a higher concentration of cyanidin glycosides content, compared to both lettuce under UV-A and the control treatment. The levels of cyanidin in leaves under the PAR+UV-AUV-B treatment was 86% and 72% higher than the level under PAR+UV-A and PAR respectively (see Figure 11).

The mature leaves showed much higher concentrations of cyanidin glycosides for all three treatments. Therefore, age was very significant, (p=0.01). The PAR+UV-A/UV-B treatment had the highest difference in concentration between the mature and young leaves. The mature leaves had 66.7% higher concentration than the young leaves on the PAR+UV-A/UV-B and PAR+UV-A treatment, respectively. The PAR+UV-A and PAR treatment gave similar concentrations in the young leaves.

Table 4

Output from the full model Analyse of variance (GLM) effects of the UV treatments on the synthesis of cyanidin glycosides

Source	DF	F-value	P-value	Significance
Treatment	2	28.76	<0.001	***
Age	1	45.15	<0.001	***
Experiment	1	12.89	<0.001	***
Treatment*Age	2	0.83	0.441	NS
Treatment*Experiment	2	0.20	0.817	NS
Age*Experiment	1	0.00	0.965	NS
Error	68			

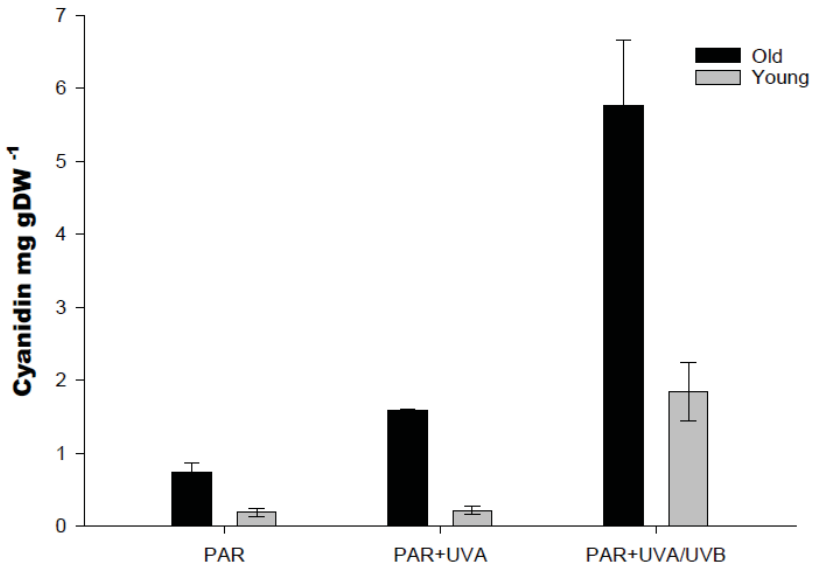

Figure 11. Concentration of Cyanidin related to dry planter material (DW) of 'Lollo Rosso' treated for 7 days under different light conditions Control (PAR) UV-A (PAR+UV-A) and UV-A+B (PAR+UV-A/UV-B). The error bars show mean±standard error.

Lettuce photos (see Appendix A red lettuce photos) taken after the three treatments reveals a strong red coloration in the leaves treated under UV-A/UV-B radiation whereas the PAR (Control) leaves were green. In fact, the red pigmentation in 'Lollo Rosso' is mainly due to the accumulation of cyanidin glycosides (Marin et al., 2015).

3.3.2. Quercetin and Quercetin glucosides

It's well known that quercetin and quercetin glucosides concentration increases in response to UV radiation (see Table 5). In fact, the results presented in the figures show that at the level of quercetin in 'Lollo Rosso' increased sharply under UV exposure (see Figure 12). The highest quercetin concentration was detected under the PAR+ UV-A/UV-B treatment up to 16mg gDW^{-1}. There was 70% and 80% more than in the PAR+UV-A and PAR treatments respectively. The PAR treatment showed the lowest concentration in both types of quercetin. The concentration of quercetin is markedly higher on the mature leaves (p=0.01). The PAR+UV-A/UV-B treatment gave a 65% higher concentration in the mature leaves than in the young leaves. The PAR+UV-A treatment gave 70% higher concentration in the mature leaves than in the young leaves.

Table 5

Output from the full model Analyse of variance (GLM) effects of the UV treatments on the synthesis of quercetin

Source	DF	F-value	P-value	Significance
Treatment	2	98.15	<0.001	***
Age	1	95.61	<0.001	***
Experiment	1	2.08	0.153	NS
Treatment*Age	2	1.13	0.33	NS
Treatment*Experiment	2	0.19	0.83	NS
Error	68			

Quercetin glucosides synthesis under UV radiation had the same patterns of quercetin (see Table 6). However, quercetin glucosides had lower concentrations up to 5mg gDW^{-1} under the PAR+UV-A/UV-B treatment. The PAR+UV-A treatment had a higher concentration than PAR treatment but much lower than the PAR+UV-A/UV-B treatment.

Table 6

Output from the full model Analyse of variance (GLM) effects of the UV treatments on the synthesis of quercetin glucosides

Source	DF	F-value	P-value	Significance
Treatment	2	63.07	<0.001	***
Age	1	89.47	<0.001	***
Experiment	1	3.36	0.071	*
Treatment*Age	2	4.72	0.012	*
Treatment*Experiment	2	0.4	0.672	NS
Age*Experiment	1	0.82	0.37	NS
Error	68			

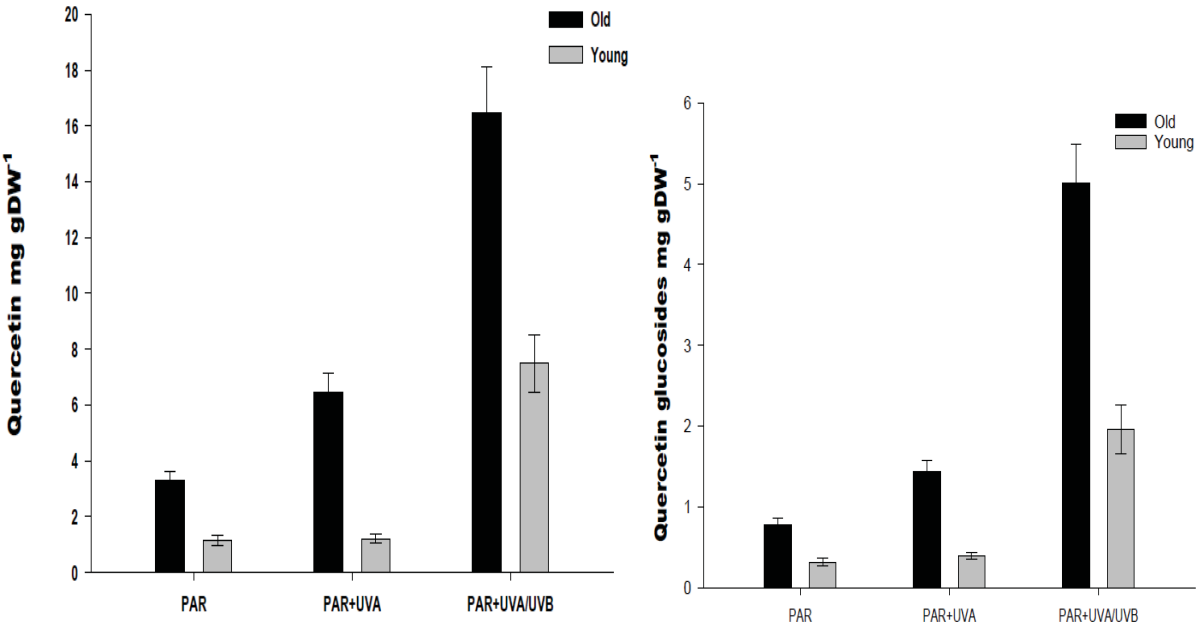

Figure 12. Total of quercetin and quercetin glucosides related to dry planter material (DW) of 'Lollo Rosso' treated for 7 days under different light conditions Control (PAR), UV-A (PAR+UV-A) and UV-A+B (PAR+UV-A/UV-B). The error bars show mean±standard error

3.4. Phenolic Compounds

3.4.1. Chicoric acid

According to the results (see figure 13) the concentration of chicoric acid was highest under the PAR+UV-A/UV-B treatment. The PAR+UV-A and the PAR treatment had respectively 43% and 58% less chicoric acid concentration than the PAR+UV-AUV-B treatment. However, this is not significant statistically. The mature leaves had the highest concentration of chicoric acid, whereas the concentration in the young leaves (see Table 7). The mature leaves in the PAR+UV-AUV-B treatment gave 38.65% more concentration than the younger leaves. In the PAR+UV-A treatment the mature leaves had 45,5% more concentration than the younger leaves. Finally, the PAR treatment gave 33.4% more concentration in the mature leaves.

Table 7

Output from the full model Analyse of variance (GLM) effects of the UV treatments on the synthesis of The synthesis of chicoric acid

Source	DF	F-value	P-value	Significance
Treatment	2	1.98	0.147	NS
Age	1	4.77	0.032	*
Experiment	1	0	0.95	NS
Treatment*Age	2	0.43	0.654	NS
Treatment*Experiment	2	0.52	0.595	NS
Age*Experiment	1	1.16	0.285	NS
Error	68			

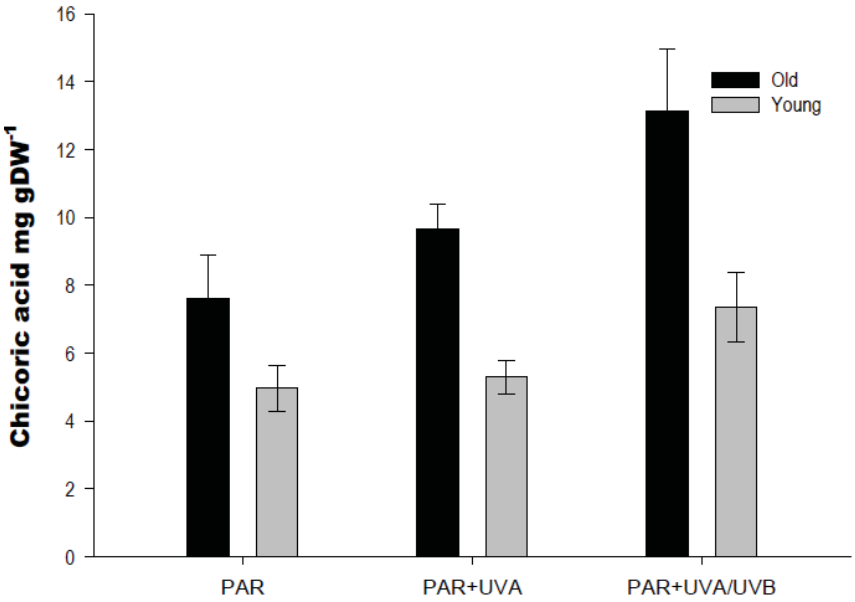

Figure 13. Concentration of chicoric acid (di-O-caffeoyltartaric acids) related to dry planter material (DW) of 'Lollo Rosso' treated for 7 days under different light conditions Control (PAR), UV-A (PAR+UV-A) and UV-A+B (PAR+UV-A/UV-B). The error bars show mean±standard error of error

3.4.2. Chlorogenic acids

In the HPLC chromatograms there was identified three chlorogenic acids with different concentrations**.** For the first chlorogenic acid identified (see Figure 14), the PAR+UV-A/UV-B treatment had 18% and 40% more chlorogenic acid than PAR+UV-A and PAR respectively. This concentration was higher in the mature leaves in all three treatments. Therefore, age is significant factor (see Table 8). The PAR+UV-A/UV-B treatment gave 50% higher concentration in the mature than in the young leaves. The PAR+UV-A treatment had 71.43% more concentration in the mature leaves and the PAR had 66.7% more concentration in the mature leaves than the young leaves. This first chlorogenic detected had the highest concentration than the others two.

Table 8

Output from the full model Analyse of variance (GLM) effects of the UV treatments on the synthesis of chlorogenic acid

Source	DF	F-value	P-value	Significance
Treatment	2	2.52	0.088	*
Age	1	26.55	<0.001	***
Experiment	1	3.18	0.079	*
Treatment*Age	2	0.52	0.595	NS
Treatment*Experiment	2	1.12	0.331	NS
Age*Experiment	1	0.09	0.763	NS
Error	68			

The second chlorogenic acid was represented in a small amount in the three treatments but have the same pattern as the first (see Figure 14). The PAR+UV-A/UV-B and PAR+UV-A treatment had the almost the same concentration showing a small difference between the three. These concentrations was higher in the mature leaves, therefore age was a factor significant (see Table 9). The control had less accumulation of Chlorogenic acid derivate than the other treatments.

Table 9

Output from the full model Analyse of variance (GLM) effects of the UV treatments on the synthesis of chlorogenic acid

Source	DF	F-value	P-value	Significance
Treatment	2	5.06	0.009	**
Age	1	38.65	<0.001	***
Experiment	1	2.02	0.159	NS
Treatment*Age	2	1.09	0.343	NS
Treatment*Experiment	2	0.26	0.775	NS
Age*Experiment	1	0.03	0.873	NS
Error	68			

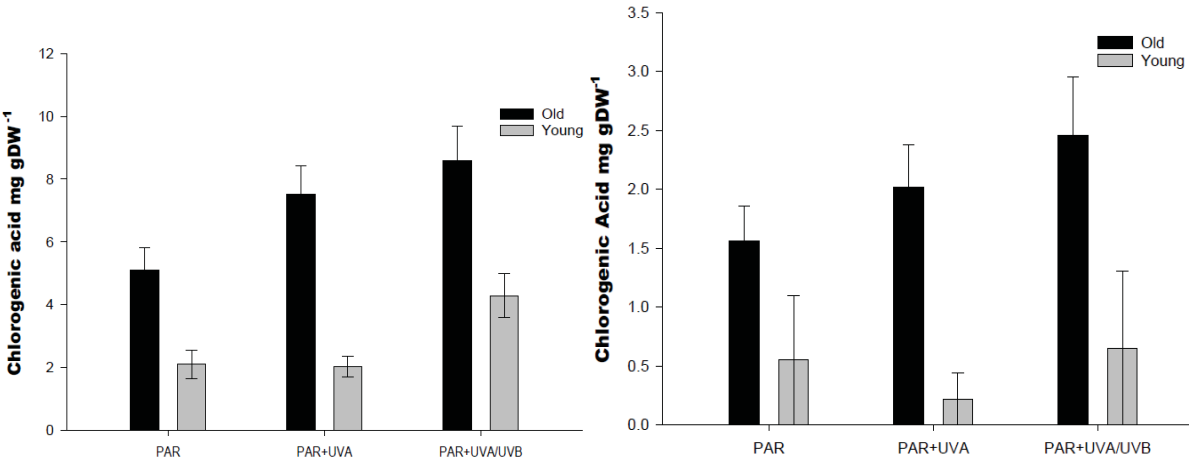

Figure 14. Concentration of chlorogenic acid (5-o-caffeoylquinic acid) related to dry planter material (DW) treated for 8 days under different light conditions Control (PAR), UV-A (PAR+UV-A) and UV-A+B (PAR+UV-A/UV-B). The error bars show mean±standard error of error

The third chlorogenic acid had the lowest concentration in the three treatments (see Figure 15). The treatments were not significant; the PAR+UV-A had the highest concentration, followed by the PAR treatment. In contrast to the two other chlorogenic acids found, this one showed a higher concentration in the young leaves in the three treatments (see Table 10).

Table 10

Output from the full model Analyse of variance (GLM) effects of the UV treatments on the synthesis of chlorogenic acid

Source	DF	F-value	P-value	Significance
Treatment	2	1.6	0.209	NS
Age	1	73.99	0.001	***
Experiment	1	3.4	0.07	*
Treatment*Age	2	1.1	0.339	NS
Treatment*Experiment	2	1.42	0.248	NS
Age*Experiment	1	4.89	0.03	*
Error	68			

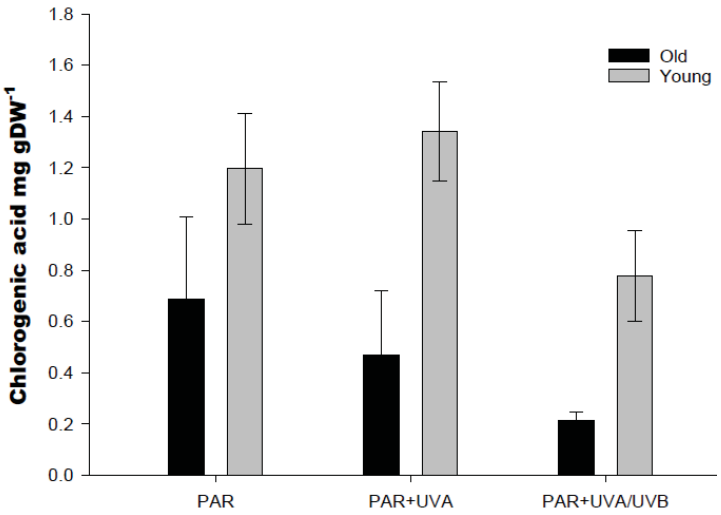

Figure 15. Concentration of chlorogenic acid (5-o-caffeoylquinic acids) related to dry planter material (DW) of 'Lollo Rosso' treated for 7 days under different light conditions Control (PAR), UV-A (PAR+UV-A) and UV-A+B (PAR+UV-A/UV-B). The error bars show mean±standard error of error.

3.5. Results of the Sensorial Analysis

There was found significant variation in the results. The participants were able to identify differences between the red lettuces tested. The PAR treatment was reported be the best as well being the least bitter lettuce. The PAR+UV-A/UV-B salad was reported the most bitter as well being the least liked lettuce (see Figure). The PAR treatment was reported the best as well being the least bitter lettuce. The PAR+UV-A/UV-B salad was reported the most bitter as well being the least liked lettuce (see Figure 16).

3.5.1. Results for the best taste

The results for the best red lettuce according to the participants preference was statistical significant (ρ=0.0089). A total of 19 out 33 participants reported that the best lettuce was from the PAR treatment. While 5 out 33 participants reported the best lettuce was from the PAR+UV-A/UV-B treatment. Further, 9 out 33 participants reported PAR+UV-A was the best red lettuce

3.5.2. Results for the most bitter taste

The results when using the sample data set for bitter taste are not strong enough to conclude that there is a statistically significant (ρ=0,1482). Participants reported that PAR+UV-A/UV-B treated lettuce was the most bitter with 15 out 33. While 6 out of 33 reported that the control was most bitter. And 12 out 33 reported that PAR+UV-A was most bitter red lettuce.

The PAR treatment was reported to be the best as well being the least bitter red lettuce. The PAR+UV-A/UV-B red lettuce was reported the most bitter as well being the least liked lettuce.

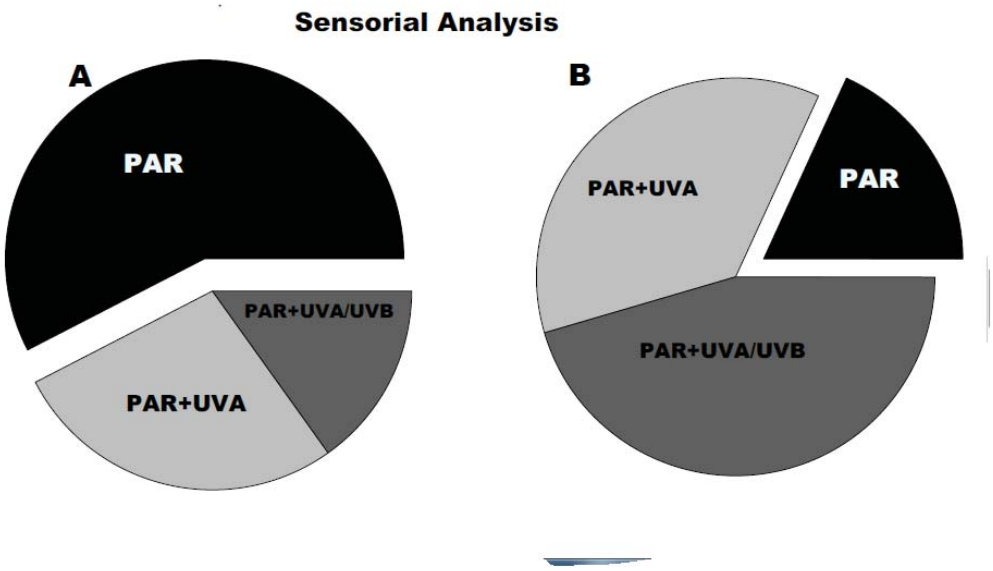

Figure 16. Displays the preference of three types of 'Lollo Rosso' in sensorial analysis; Control (PAR), UV-A (PAR+UV-A) and UV-A+B (PAR+UV-A/UV-B) A) for the red lettuce with best taste B) for red lettuce with most bitter taste

4. Discussion

4.1. Stomatal Conductance Response to UV Radiation in Red Lettuce

Previous studies have shown that stomata movements are highly related to UV radiation and this is well documented (Jansen and Van Den Noort, 2000, Giannini et al., 1996, Bornman and Teramura, 1993, Sullivan and Teramura, 1988, Musil and Wand, 1993, Nogués et al., 1999, He et al., 2005, Wu et al., 2012, Christie et al., 2012, Tossi et al., 2014). UV radiation has been reported to stimulate both stomatal opening and closure depending on wavelengths (He et al., 2005, Zeiger, 1983, Negash and Björn, 1986, Wargent et al., 2009). UV-A stimulates opening of stomata and consequently stomatal conductance (Jansen and Van Den Noort, 2000). UV-B radiation was reported to increase stomatal conductance in cucumber (Teramura et al., 1983) and some species of *Ericaceae* (Musil and Wand, 1993). However, results of an experiment with species of pea, grown under UV-B in greenhouse conditions, showed a decrease in stomatal conductance (Jansen and Van Den Noort, 2000)

Indeed, both types of UV-A and UV-B wavelength can, according to the literature, regulate stomatal conductance through different pathways (Jansen and Van Den Noort, 2000, Kostina et al., 2001, Tossi et al., 2014, Musil and Wand, 1993, He et al., 2005)**.** UV-A stimulates stomata opening, consequently increasing stomatal conductance, which is mediated by the blue light photoreceptor (Brazaitytė et al., 2015). UV-B on the other hand affects stomata in a more complex way both in opening and closing, but is clearly more related to the stomatal closure and lower conductance (Nogués et al., 1999, Jansen and Van Den Noort, 2000).

One objective of this study was to evaluate the use of supplementary light in greenhouses and the effects on stomatal conductance. The hypothesis that the different types of UV radiation affect stomatal conductance in different ways has been refuted. The result present in the figures (see Figure 10) shows that there is no significant difference in stomatal conductance in red lettuce under the different treatments used when comparing the average values from the whole experiment (PAR, PAR+UV-A and PAR+UV-A/UV-B). These results agree with the study conducted on red lettuce by Tsormpatsidis et al. (2010), although they used a different approach in their fieldwork. In their study they used UV blocking filters and the plants were exposed to different UV conditions from the growing period.

However, a different response to UV radiation was seen depending on leaf side and leaf age (see figure 10 A and B). Generally, stomata are present on both the upper and lower side of the leaves of lettuce but a higher conductance was measured on the upper side of the expanded leaves (see Figure 10A). The upper side of the leaves get a higher level of light incidence than the lower side (Lawson, 2009). Since, the light comes from above and reaches the upper layers of the lettuce. This may result in higher conductance since light is an important signal for opening (Shimazaki et al., 2007). As described above, the stomatal conductance response to UV-A exposure is more related to stomatal opening and the stomatal conductance to UV-B tends to induce stomatal closure. In this study was observed that the PAR and PAR+UV-A showed a slightly higher conductance compared with the PAR+UV-A/UV-B treatment (see Figure 10A). In fact, the Figure 10A also shows the leaves treated with PAR+UV-A/UV-B had the lowest stomatal conductance. However the changes in stomatal conductance are small and probably not important in a production perspective. High transpiration postharvest can be a problem in many leafy vegetables but UV exposure seems not have a prominent role in this experimental set-up.

The mature leaves had a higher stomatal conductance than the young leaves and this may be explained by the leaves' development. The mature leaves are important for photosynthesis, are more developed than the younger ones, and a higher stomatal conductance may be linked with the increase in stomata size and density in mature leaves. Kostina et al. (2001) found a considerable increase in stomatal conductance in Betula pendula after a longer period of exposure to natural UV radiation when the leaves were more developed.

4.2. Effects of UV Radiation on Synthesis of Phenolic Acids Flavonoids

The results of this study revealed that the synthesis of phenolic acids and flavonoids increased considerably under UV radiation. There was an overall trend to a higher compound concentration in response to UV radiation compared to the PAR treatment. UV radiation is known to induce the synthesis of plants' secondary compounds. The synthesis of phenolic acids and flavonoids is found to give plants protection against UV stress (Winkel-Shirley, 2002, Hagen, 2006, Tossi et al., 2011). These results are in line with research (Wilson et al., 2001, Tsormpatsidis et al., 2008) that has reported an overall high accumulation of phenolic acids and flavonoids in response to UV radiation. Quercetin and cyanidin glycosides are often the most abundant, though in this study in Lollo Rosso it was flavonoids. These results are

consistent with García-Macías et al. (2007) , Behn et al. (2011) and Crozier et al. (1997), who have all reported much higher quercetin concentrations in red lettuce.

There was expected to see an increase of luteolin conjugates concentrations as was found previously (DuPont et al., 2000, García-Macías et al., 2007). Luteolin conjugates considerably increase in concentration in response to UV radiation exposure. Moreover, Oh et al. (2009) evaluated the increase of phenolic compound concentrations in lettuce cv. Baronet. In that study, luteolin concentration increased considerably under light stress. However, according to the results in this study luteolin was not identified, which may be due to very much lower concentrations in these samples, making luteolin difficult to detect. Some other studies have also failed to detect this compound Crozier et al. (1997) and Hertog et al. (1992). Another explanation may be the different approaches in the present study and García-Macías et al. (2007) (fieldwork done using filters to block UV radiation). According to Romani et al. (2002) flavonoid content in general tends be higher in plants cultivated under field conditions than in plants cultivated in greenhouses. This may be explained by the absorption of UV by greenhouse glass, which are otherwise present in solar radiation in the field.

4.3. Effects of UV Radiation on Flavonoids Synthesis

4.3.1. Cyanidin glycosides

Cyanidin glycosides showed a significant increase in the different UV treatments. Lettuce under the PAR+UV-A/UV-B treatment had the highest concentration, followed by the PAR+UV-A (see Figure 11). These results are in line with the study of Tsormpatsidis et al. (2008). Although they used a different approach in their study (fieldwork using filters to block different UV radiation), the authors showed that anthocyanin content was greatest when PAR+U-VA/UV-B were present. When UV-B was excluded and UV-A was transmitted, anthocyanins concentrations were reduced, and even lower concentrations were found when both UV-A and UV-B were excluded. These results are partially supported by the study of (Li and Kubota, 2009). Li and Kubota (2009), by using different frequencies of light, demonstrated that the supplement of UV-A increased the accumulation of anthocyanin.

The role of cyanidin glycosides in plants is still a topic of discussion, but it has been proposed that they act as UV-B shield and are a powerful antioxidant. Accumulation of these compounds in plants may help them protect themselves by mitigating photoinhibition as well

as DNA damage (Behn et al., 2011, Becker et al., 2014b). They may do this by attenuating the green light, shielding the chloroplasts against excess radiation and acting as a ROS scavenger (Neill and Gould, 2003).

In general, the cyanidin glycosides concentration varies with leaves' development. I the current study this concentration was higher in the mature leaves compared with the younger leaves. This is not in agreement with studies (Becker et al. (2014b) and Behn et al. (2011)) of cyanidin glycosides levels in mature leaves compared to other growth stages. In the Becker et al. (2014b) study, plants were harvested in 3 different stages, 12, 21 and 35 days after planting. These plants were continuously exposed to natural UV radiation in a greenhouse. The cyanidin glycoside level was higher in the phase between pre-heading and heading than later between the heading and mature heads. Behn et al. (2011) has found that cyanidin glycosides increased in concentration in young leaves following a short UV-B radiation exposure. In this study the plants were cultivated for 20 days and then transferred to UV-B radiation treatment for 2 days. The short exposure lead to a higher cyanidin concentration in the young leaves only.

The results are not in agreement with these two studies Behn et al. (2011) and Becker et al. (2014b). They reported higher concentrations of cyanidin glycosides in mature leaves. However, there are many differences between these two studies and in the current investigation. In this research these concentrations was evaluated in young and mature leaves in the same plant. In fact, there was not assessed the concentration of phenolic compounds and flavonoids at a very early stage. The plants were cultivated for 30 days before being subjected to seven days of different UV treatments. The plants were submitted to a longer treatment and the plants were older compared with both studies Behn et al. (2011); Becker et al. (2014b).

According to Hohl et al. (2001) cyanidin glycosides synthesis is light dependent, meaning that the plant needs to be directly exposed to light in order to induce synthesis. The young leaves may not have been directly exposed to light due their position on the plant. Once the mature leaves were bigger and wide enough to block the light, the younger leaves had lower accumulation. Furthermore, the synthesis of cyanidin glycosides also occurs through the phytochromes as high-irradiance responses. That may mean it is necessary to have a longer or a continuous exposure to light of relatively higher radiance to induce the response (Taiz, 2010, Mancinelli and Rabino, 1975). In the results reported by Behn et al. (2011), the short

period of induction might not have been long enough to induce the cyanidin synthesis in all leaves. In the experiment of Becker et al. (2014b), supplementary light was not used. This means there was only the low level of natural radiation.

4.3.2. Quercetin and Quercetin glucosides

The amount of quercetin was higher than of the other compounds analysed. Crozier et al. (1997) had also found a higher accumulation of quercetin in Lollo Rosso. These concentrations had similar trends to cyanidin glycosides and increased synthesis under the PAR+UV-A/UV-B treatment. According to García-Macías et al. (2007), Behn et al. (2011) and Becker et al. (2014b) supplementary UV-B radiation increased quercetin concentrations and this is confirmed in my study. The PAR+UV-A radiation treatment also had an effect on increase of synthesis quercetin but was lower than the PAR+UV-A/UV-B together (see Figure 12).

In regard to the plant age, the mature leaves had a higher accumulation than the younger leaves in the three treatments, and this is in line with (Behn et al., 2011). According to Behn et al. (2011) and Becker et al. (2014a) quercetin synthesis tend to increases in the all leaf stage, while Romani et al. (2002) suggested that the amount of quercetin are higher in young leaves compared to the mature leaves. It seems that there is no clear agreement concerning quercetin concentration and leaf stage, but it may also be due to the different focus and techniques used in the different studies. The main focus of my project was to study the differences between the three treatments applied. There was to evaluate the effects of distinct UV radiation on synthesis of phenolic compounds and flavonoids in red lettuce. The results presented are supported by the results of previous studies (García-Macías et al., 2007, Tsormpatsidis et al., 2008, Behn et al., 2011)

4.4. Effects of UV Radiation on Synthesis of Phenolic Acids

The main phenolic acids found in were chicoric acid and chlorogenic acids. In this research caffeic acid was not detected in the HPLC analysis. These compounds showed a significant increase in concentration in the three treatments. According to (Llorach et al., 2008) chicoric acid and chlorogenic acids are the main phenolic compounds in red lettuce.

UV radiation had been reported to increase the overall concentration of phenolic acid in red lettuce (García-Macías et al., 2007, Tsormpatsidis et al., 2008). In fact, overall the light

treatments caused a significant increase in chicoric acid. All three treatments had a positive effect on the concentration of chicoric acid. Although the PAR+ UV-A/UV-B treatment showed the highest concentration of chicoric acid, there is no significant difference in effect between the treatments applied (see Figure 13). This is in line with previous studies that have reported a higher chicoric acid concentration in lettuce (Romani et al., 2002, Oh et al., 2009, García-Macías et al., 2007).

Regarding chlorogenic acid, the concentration was higher under the PAR+ UV-A/UV-B treatment. The first chlorogenic acid detected had the highest concentration, and showed no significant difference in concentration between the three treatments (see Table 8). The other chlorogenic acids were only present in small amounts and none showed significant differences across the different treatments (see Figure 14 and 15). Overall, chlorogenic acid showed the same trends as chicoric acid but at a lower concentration. In the current research chlorogenic acid content was affected by all the treatments but a slight higher concentration in the UV-B radiation (see Figure 14). These results are in agreement to Tegelberg et al. (2004) chlorogenic acid synthesis increases under UV-B radiation. In that study they compared R, FR, R+UV-B and FR+UV-B and had a higher chlorogenic acid concentration under the FR+UV-B. Oh et al. (2009) also reported a higher synthesis of chlorogenic acid under higher light stress.

In regard to plant age, in overall the mature leaves had a higher concentration of chlorogenic acid. Therefore, age was a actor significant (see Table 8 and 9). A higher chlorogenic acid concentration in matures leaves might explain their antioxidant functions in the plant. Tamagnone et al. (1998) demonstrate the limitation of chlorogenic acid lead to a cell death in mature leaves of tobacco. The last chlorogenic acid detected gave the lowest concentration and surprising a higher concentration in the young leaves.

4.5. The Effects of UV Radiation in Colour and Taste of Red Lettuce

Phenolic compounds and flavonoids are known to be the main chemicals responsible for the colour and taste of red lettuce. In addition to its attractive red colour, the ‘Lollo Rosso’ variety of red lettuce is a widely accepted source of antioxidants, due to the antioxidant proprieties of the phenolic compounds and flavonoids it contains (Oh et al., 2009, García-Macías et al., 2007, Becker et al., 2013). The attractive red colour of this lettuce is an important aspect that can affect people's perception of higher nutrition and commercial value.

UV radiation is reported by previous studies to increase the cyanidin glycoside concentration in 'Lollo Rosso' (Tsormpatsidis et al., 2008, Behn et al., 2011, Becker et al., 2014b, Marin et al., 2015). Although other pigments such as chlorophylls and carotenoids also contribute to the colour of 'Lollo Rosso' (Marin et al., 2015), the red colour is mainly due to the concentration of cyanidin glycosides in its leaves (Harborne, 2013).

The results showed at the PAR+UV-A/UV-B treatment produced a stronger red colour than the PAR+UV-A and PAR respectively (Appendix A photos). This red colour was positively related to the higher cyanidin concentration on the HPLC analyses. The present results are agreement with (García-Macías et al., 2007, Tsormpatsidis et al., 2008) who also found higher cyanidin glycoside concentrations in lettuce cultivated under film, which allowed exposure to UV radiation.

In this study taste of the red lettuce treated with the three treatments was also evaluated (PAR, PAR+UV-A, PAR+UV-A/UV-B). Many studies have analysed the concentration of phenolic acid and flavonoids in plants cultivated or treated with UV radiation but few studies have evaluated the effects of these compounds on taste. The objective was to evaluate the bitter taste of the red lettuce following the three treatments. Further, it was also of interest to evaluate the relationship between the flavour of the red lettuce and the content of the main phenolic acids and flavonoids that may the cause the bitter taste.

Quercetin are flavonols found in red lettuce that known to have a bitter taste (Drewnowski and Gomez-Carneros, 2000, Olthof et al., 2001). In addition the phenolic compound chlorogenic acid may have a bitter taste. Red lettuce also contains other compounds that may promotes taste bitter. According to Price et al. (1990) lactucin glycoside is thought to increase the bitter taste in lettuce. Furthermore according to the authors mentioned above, 'Lollo Rosso' has the highest bitterness score amongst the lettuces analysed but not the highest amount of compound that are thought to cause the bitter taste. That means that red lettuce may contain other types of compound that contributes to its bitter taste. Tamaki et al. (1995) found a high level of sesquiterpene lactones in three wild species of Lactuca Sativa. However, such compounds have been genetically targeted and reduced in domestic lettuce (Chadwick et al., 2016).

The results of the sensory analysis presented in the figures (see Figure 16 A and B) showed that the participants' perceptions correlated with the chemical analysis. The PAR treatment was reported to result in the best taste and according the results of the chemical analyses had

the lowest total amount of phenolic acids and flavonoids. On the other hand, the PAR+UV-A/UV-B treatment was commensurate with a stronger bitter taste, and produced the highest content of all phenolic acid and flavonoids in the chemical analysis. Quercetin was the main flavonoid present in the PAR+UV-A/UV-B treatment, and this flavonol is known to have a bitter taste (Drewnowski and Gomez-Carneros, 2000). This compound is for example suggested to cause the bitter taste in immature apples (Drewnowski and Gomez-Carneros, 2000), and is in fact the most common flavonol present in the apples' skin (Best, 2012). Moreover, according to Roland et al. (2013), flavonols have a chemical group and structure which is typical of bitter compounds.

The red lettuce treated with the PAR+UV-A/UV-B gave a stronger bitter taste than the other treatments. The leaves used in this experiment were a random selection from plants that were cultivated for 38 days. This result is similar to Eskins et al. (1995), who evaluated the effect of light on the bitter taste intensity of mature red lettuce. In their study the authors concluded that leaves grown under white light had a stronger bitter taste, and the bitterness of these leaves increased with leaf maturity. However, they did not evaluate the chemical content of the leaves as was assess in the present study.

Although chlorogenic acid is the main phenolic acid in coffee (Feldman et al., 1969) this compound does not seem to contribute to its bitter taste. The same may be true for the red lettuce grown in my study where the three treatments gave a significant increase in chlorogenic acid content. The PAR treatment did not seem to lead to a bitter taste in the sensory analyses. Furthermore, according to (Nagel et al., 1987) chlorogenic acid is not a bitter compound.

4.6. Implications of UV Radiation in Greenhouse Production

In Norway, the use of supplementary light in greenhouses allows production of crops all year round. During the winter, the low level of natural sunlight is the main factor limiting crop production. Many studies have been conducted to compare the effects of different types of artificial light in crop management. The common HPS lamp type is used to increase photosynthesis and growth in greenhouses. However, they contain very little blue light and no UV radiation. Processes like anthocyanin synthesis and colour development are supressed in such light environment (Rodriguez et al., 2014). UV fluorescent lamps may have an effect on leaf shape and colour but have less of an effect on photosynthesis and plant growth (Moe et

al., 2005, Grimstad, 1982). In fact, recent research showed that plants under UV radiation can respond by accumulating secondary compounds such as phenolic acids and flavonoids (Wilson et al., 2001, Helsper et al., 2003, Jenkins, 2014, Štroch et al., 2015). Since phenolic acids and flavonoids absorb light at different wavelengths (Yao et al., 2004) this may explain the need for full spectrum lighting to increase the accumulation of both these compounds in plants. According to Wilson et al. (2001) flavonoid synthesis increases in response to UV radiation. Thus, a combination of HPS and UV fluorescent tubes might be useful in commercial production to optimize photosynthesis and induces synthesis of secondary compounds. A short exposure period in the end of the production with UV fluorescent tubes can be a useful methods for growers to ensure red colour and high accumulation of healthy compounds in the periods with low natural light.

Moreover, the stomata movements in plants also depend on the wavelength of light that they are exposed to (Wargent et al., 2009). The active spectrum for stomatal opening was reported by (Eisinger et al., 2000). Here, the authors showed that stomata opening occurs when plants are irradiated with light in the spectrum from 280nm to 360nm. According to Negash and Björn (1986), wavelengths of 285nm and shorter are the most in inducing stomatal closure. In the presented experiment only small effects of UV was observed on stomatal conductance indicating that treatments with rather low levels of UV-A and UV-B are less important in determining transpiration of 'Lollo Rosso'.

Generally, the fluorescents lamps UVB-313 and the UVA-340 are used in research work in greenhouses. These UV lamps have distinct spectra and emit different quantities of energy (Q-LAB). In this study, we employed UVA-340 lamps. These lamps emit a broad spectrum of light analogous to sunlight. The UVB-313 lamp is often used to increase the UV-B level in UV-B research experiments. Unfiltered UV-B has radiation down to 275 nm and may also control the build-up of powdery mildew (Suthaparan et al., 2016). However, the shortwave UV-B light is dangerous for people working in the greenhouse. The present study shows that the UVA -340 lamps are efficient at increasing phenolic compounds in lettuce, and are much safer to work with since the UV-B radiation produced does not have shorter wavelengths than natural solar radiation. However, this longer wavelength radiation offers no protection against powdery mildew (Suthaparan et al., 2016).

4.7. Concluding Remarks

This study demonstrates that UV radiation may be a good environmental tool in greenhouses to stimulate synthesis of phenolic acids and flavonoids. I am able to confirm the hypothesis that the synthesis of phenolic acids and flavonoids in red lettuce is affected by radiation. The concentrations of all compounds analysed showed a considerable increase under the PAR+UV-A/UV-B treatment. This means that the production of red lettuce with higher concentrations of these compounds (phenolic acid and flavonoids), which are considered beneficial for human health, could be achieved using supplementary light as in this study.

In this research the second hypothesis, UV radiation affect the taste of red lettuce was confirmed. Unfortunately, the PAR+UV-A/UV-B treatment also produced a stronger bitter tasting in 'Lollo Rosso'. A further understanding on how to minimise the undesirable bitter taste in red lettuce treated with full-spectrum light is now needed. I suggest that future studies on red lettuce use a shorter period of UV treatment, to determine whether a 4-day UV treatment might be able to reduce the bitter taste without a reduction in production of the healthy chemical compounds. I also recommend a more detailed analysis of the compounds that may be the major causes of the bitter taste in red lettuce. One could also to test to see whether the bitter tasting compounds also increase in response to the treatments applied. Finally, we could use a trained panel as a control to produce more reliable sensory results (Ley, 2008). The bitter taste in red lettuce is an undesirable factor and may lead to a lower acceptability amongst consumers.

The third hypothesis was refuted. Although the use of UV radiation is reported to affect stomatal conductance. However, there was a trend towards lower stomatal conductance under the PAR+UV-A/UV-B treatment in both expanded and non-expanded leaves compared to control and UV-A treatments. To achieve a lower stomatal conductance in red lettuce is a factor desirable to preserve the appearance, texture and the crispiness of the lettuce post harvest. As a practical implication this study suggests that the use of UV-B to induce anthocyanins synthesis also can reduce stomatal conductance and may be significant factor linked to lower transpiration in greenhouses production. However, the relevance of this in commercial production needs to be tested further.

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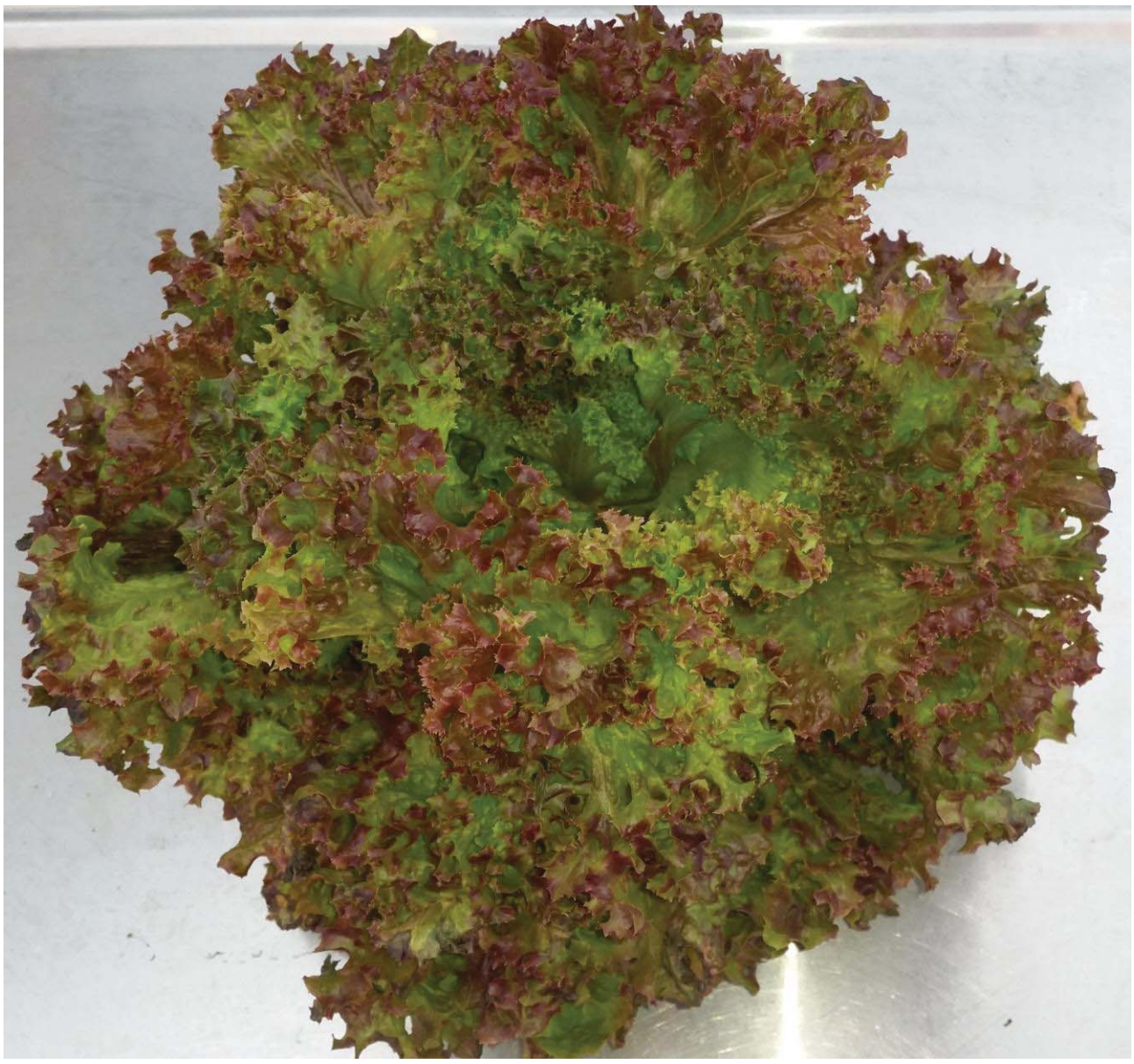
Appendix A - Red lettuce photos taken after the UV treatments

Photos of the red lettuce from the PAR (Control) treatment

Appendix A

Photos of the red lettuce from the UV-A (PAR+UV-A) treatment



Appendix A

Photos of the red lettuce from the UV-A+B (PAR+UV-A\UV-B) treatment

Appendix A

Appendix B

Stomatal Conductance Measurements – Young Leaves

The original data frames required for the stomatal conductance measurement in young non-expanded leaves in red lettuce.

Side	UV	Experiment	AVG
Upper	Control	First	102.0
Upper	Control	First	136.5
Upper	Control	First	178.0
Upper	Control	First	205.0
Upper	Control	First	219.5
Upper	Control	First	160.5
Upper	Control	First	140.0
Upper	UVA	First	105.5
Upper	UVA	First	129.5
Upper	UVA	First	385.0
Upper	UVA	First	207.0
Upper	UVA	First	223.5
Upper	UVA	First	198.5
Upper	UVA	First	124.5
Upper	UVA/UVB	First	99.0
Upper	UVA/UVB	First	124.5
Upper	UVA/UVB	First	147.5
Upper	UVA/UVB	First	221.5
Upper	UVA/UVB	First	115.5
Upper	UVA/UVB	First	118.0
Upper	UVA/UVB	First	124.5

Appendix B

Side	UV	Experiment	AVG
Upper	Control	Second	68.5
Upper	Control	Second	103.0
Upper	Control	Second	148.5
Upper	Control	Second	77.5
Upper	Control	Second	115.5
Upper	Control	Second	204.0
Upper	Control	Second	182.0
Upper	UVA	Second	290.0
Upper	UVA	Second	287.0
Upper	UVA	Second	277.0
Upper	UVA	Second	181.0
Upper	UVA	Second	138.0
Upper	UVA	Second	80.5
Upper	UVA	Second	155.5
Upper	UVA/UVB	Second	68.5
Upper	UVA/UVB	Second	99.0
Upper	UVA/UVB	Second	72.5
Upper	UVA/UVB	Second	162.5
Upper	UVA/UVB	Second	182.0
Upper	UVA/UVB	Second	63.5
Upper	UVA/UVB	Second	84.5
Lower	Control	First	105.5
Lower	Control	First	232.0
Lower	Control	First	293.0
Lower	Control	First	239.0
Lower	Control	First	160.0
Lower	Control	First	157.0
Lower	Control	First	134.5
Lower	UVA	First	143.5

Appendix B

Side	UV	Experiment	AVG
Lower	UVA	First	242.5
Lower	UVA	First	105.5
Lower	UVA	First	90.5
Lower	UVA	First	192.0
Lower	UVA	First	138.0
Lower	UVA	First	169.5
Lower	UVA/UVB	First	161.5
Lower	UVA/UVB	First	145.0
Lower	UVA/UVB	First	141.5
Lower	UVA/UVB	First	275.0
Lower	UVA/UVB	First	258.0
Lower	UVA/UVB	First	100.0
Lower	UVA/UVB	First	131.5
Lower	Control	Second	258.0
Lower	Control	Second	76.5
Lower	Control	Second	84.0
Lower	Control	Second	36.0
Lower	Control	Second	122.5
Lower	Control	Second	131.0
Lower	Control	Second	72.0
Lower	UVA	Second	50.0
Lower	UVA	Second	62.0
Lower	UVA	Second	82.0
Lower	UVA	Second	80.5
Lower	UVA	Second	119.0
Lower	UVA	Second	154.5
Lower	UVA	Second	77.5
Lower	UVA/UVB	Second	35.5
Lower	UVA/UVB	Second	114.0

Appendix B

Side	UV	Experiment	AVG
Lower	UVA/UVB	Second	96.0
Lower	UVA/UVB	Second	115.5
Lower	UVA/UVB	Second	93.5
Lower	UVA/UVB	Second	116.0
Lower	UVA/UVB	Second	235.0

Appendix C

Stomatal Conductance Measurements – Mature Leaves

The original data frames required for the stomatal conductance measurement in fully mature expanded leaves in red lettuce.

Side	UV	Repicate	AVG
Upper	Control	First	102.0
Upper	Control	First	136.5
Upper	Control	First	178.0
Upper	Control	First	205.0
Upper	Control	First	219.5
Upper	Control	First	160.5
Upper	Control	First	140.0
Upper	UVA	First	105.5
Upper	UVA	First	129.5
Upper	UVA	First	385.0
Upper	UVA	First	207.0
Upper	UVA	First	223.5
Upper	UVA	First	198.5
Upper	UVA	First	124.5
Upper	UVA/UVB	First	99.0
Upper	UVA/UVB	First	124.5
Upper	UVA/UVB	First	147.5
Upper	UVA/UVB	First	221.5
Upper	UVA/UVB	First	115.5
Upper	UVA/UVB	First	118.0

Side	UV	Repicate	AVG
Upper	UVA/UVB	First	124.5
Upper	Control	Second	68.5
Upper	Control	Second	103.0
Upper	Control	Second	148.5
Upper	Control	Second	77.5
Upper	Control	Second	115.5
Upper	Control	Second	204.0
Upper	Control	Second	182.0
Upper	UVA	Second	290.0
Upper	UVA	Second	287.0
Upper	UVA	Second	277.0
Upper	UVA	Second	181.0
Upper	UVA	Second	138.0
Upper	UVA	Second	80.5
Upper	UVA	Second	155.5
Upper	UVA/UVB	Second	68.5
Upper	UVA/UVB	Second	99.0
Upper	UVA/UVB	Second	72.5
Upper	UVA/UVB	Second	162.5
Upper	UVA/UVB	Second	182.0
Upper	UVA/UVB	Second	63.5
Upper	UVA/UVB	Second	84.5
Lower	Control	First	105.5
Lower	Control	First	232.0
Lower	Control	First	293.0
Lower	Control	First	239.0
Lower	Control	First	160.0
Lower	Control	First	157.0

Side	UV	Repicate	AVG
Lower	Control	First	134.5
Lower	UVA	First	143.5
Lower	UVA	First	242.5
Lower	UVA	First	105.5
Lower	UVA	First	90.5
Lower	UVA	First	192.0
Lower	UVA	First	138.0
Lower	UVA	First	169.5
Lower	UVA/UVB	First	161.5
Lower	UVA/UVB	First	145.0
Lower	UVA/UVB	First	141.5
Lower	UVA/UVB	First	275.0
Lower	UVA/UVB	First	258.0
Lower	UVA/UVB	First	100.0
Lower	UVA/UVB	First	131.5
Lower	Control	Second	258.0
Lower	Control	Second	76.5
Lower	Control	Second	84.0
Lower	Control	Second	36.0
Lower	Control	Second	122.5
Lower	Control	Second	131.0
Lower	Control	Second	72.0
Lower	UVA	Second	50.0
Lower	UVA	Second	62.0
Lower	UVA	Second	82.0
Lower	UVA	Second	80.5
Lower	UVA	Second	119.0
Lower	UVA	Second	154.5

Side	UV	Repicate	AVG
Lower	UVA	Second	77.5
Lower	UVA/UVB	Second	35.5
Lower	UVA/UVB	Second	114.0
Lower	UVA/UVB	Second	96.0
Lower	UVA/UVB	Second	115.5
Lower	UVA/UVB	Second	93.5
Lower	UVA/UVB	Second	116.0
Lower	UVA/UVB	Second	235.0

Appendix D

HPLC Dataframe

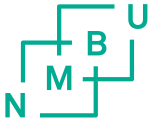
The original data frames required for the HPLC analyses of phenolic acids and flavonoids

Treatment	Experi-ment	Weight	Age	Antho-cyanin mg gDW-1	Quer-cetrin mg gDW-1	Chlorogenic acid derivate mg gDW-1	Quercetin derivate mg g DW-1	Chichoric acid mg gDW-1	Chlorogenic acid mg gDW-1	Chlorogenic acid mg gDW-1
Control	1	0.024	Young	0.3350720	1.5257072	0.2988089	0.3378081	5.3879107	0.3097632	0.3470636
Control	1	0.017	Young	0.0000000	0.7010571	0.1385348	0.2170514	3.5312879	1.4929789	1.9657323
Control	1	0.02	Young	0.0000000	0.5880714	0.0916697	0.1467377	2.3173229	0.6680854	0.8808741
Control	1	0.018	Young	0.4486800	1.8574852	0.3569599	0.5215084	7.6790728	4.0064919	0.3364660
Control	1	0.022	Young	0.7249960	2.5329992	0.6027556	0.7249124	6.8581482	5.7088586	0.3306751
Control	1	0.022	Young	0.1702327	0.9849185	0.2063157	0.2741171	4.2362731	1.2109144	1.9142560
Control	1	0.024	Young	0.4469144	1.6060151	0.4240129	0.4289181	5.6591013	2.0299757	2.0128016
Control	1	0.022	Old	0.6620173	3.3727538	1.2271834	0.6926393	7.1488591	6.7557055	0.2853698
Control	1	0.019	Old	0.6855262	3.4326978	1.6807686	0.6806759	6.7042751	5.0354142	0.2234281
Control	1	0.023	Old	0.5012380	2.6343905	1.5198929	0.5216861	0.0159127	0.7735893	0.0235712
Control	1	0.017	Old	0.5208910	2.8323129	1.1598543	0.3692656	5.7396857	5.3739962	0.2163572
Control	1	0.019	Old	1.0194701	3.6115374	1.7016923	0.9037623	8.4899065	8.4896643	0.1746262
Control	1	0.018	Old	1.9556990	5.6796149	1.5385571	0.9290882	9.7542794	8.8164242	0.2358089
UVA	1	0.021	Young	0.5548025	0.0495134	0.2756255	0.6874198	7.2629156	3.2238029	0.4066532
UVA	1	0.019	Young	0.1785185	1.3120441	0.2306206	0.3760743	5.5453751	3.1379556	0.5090998

Treatment	Experi-ment	Weight	Age	Antho-cyanin	Quer-cetrin	Chlorogenic	Quercetin	Chichoric	Chlorogenic	Chlorogenic
UVA	1	0.024	Young	0.5279498	1.5894672	0.4945489	0.3963011	5.3940134	4.1217339	0.3840692
UVA	1	0.024	Young	0.1266645	1.1734269	0.1297342	0.3028682	3.7926424	1.0597746	2.1614500
UVA	1	0.024	Young	0.1915623	1.7067971	0.1928989	0.5082852	5.5411530	1.3116180	2.3593107
UVA	1	0.023	Young	0.2883815	1.5012610	0.1831349	0.3675262	3.9137283	1.5266475	1.1207480
UVA	1	0.019	Old	1.1875154	5.2428516	2.8469411	1.4730982	7.9762743	4.0768531	0.1593986
UVA	1	0.02	Old	2.9207546	10.4785843	1.8883156	1.9477133	10.7208951	6.2285556	0.0927953
UVA	1	0.018	Old	2.1883811	6.7099102	3.1154151	1.2904192	10.4106276	6.5140957	0.1776797
UVA	1	0.024	Old	0.8683086	6.0428054	2.0923736	1.2726366	6.2063091	9.6481833	0.1682779
UVA	1	0.022	Old	2.4627636	10.5703538	2.8826538	2.1179875	10.7780395	7.9550128	0.2538645
UVA	1	0.021	Old	1.5069470	5.4929996	5.0113557	1.0265713	13.8572100	8.9299768	0.3047528
UVA/UVB	1	0.023	Young	1.7325694	8.0257403	0.3766893	1.8321347	6.7956125	2.9766702	0.7528404
UVA/UVB	1	0.024	Young	1.1351259	4.8987088	0.2445240	1.2954415	5.6118977	3.6560970	0.4590936
UVA/UVB	1	0.022	Young	4.3204030	13.6629922	0.5990802	3.5450217	12.0256442	7.6472796	0.3103451
UVA/UVB	1	0.024	Young	2.8442314	9.0310188	0.6153238	2.4014033	8.3623523	5.8050872	0.2716798
UVA/UVB	1	0.024	Young	0.1596450	1.3247332	0.0947681	0.3980406	2.7179985	0.8715248	2.1206274
UVA/UVB	1	0.022	Young	4.2042021	11.4561359	1.1720992	3.5756897	13.1253098	7.5195108	0.4024743
UVA/UVB	1	0.023	Old	8.1692918	19.8338549	1.6940154	3.9258490	13.3107973	6.2329374	0.2180887
UVA/UVB	1	0.021	Old	8.8075317	21.4943356	2.5136513	6.1216010	0.2108185	11.5891983	0.2108185
UVA/UVB	1	0.021	Old	6.0645847	19.0525756	1.6292802	4.5995271	13.9858612	6.8976168	0.2528070
UVA/UVB	1	0.02	Old	9.9946484	23.7479934	2.2126179	6.4032229	17.2434812	9.7802634	0.3324683
UVA/UVB	1	0.022	Old	2.9562377	10.5022993	6.6673404	4.1562616	19.6888922	12.0251805	0.2162650
UVA/UVB	1	0.022	Old	7.2941704	18.7758641	2.5126069	4.8384128	17.5374210	11.8910613	0.4718564
UVA/UVB	1	0.024	Old	5.7896663	16.5168956	3.9822409	8.0345131	20.0660752	7.9868011	0.2832195
Control	2	0.018	Young	0.0000000	0.6060849	0.1428546	0.1728259	2.1980345	1.3241446	2.7684909
Control	2	0.02	Young	0.0000000	0.5098736	0.1304367	0.1523139	1.7482760	1.3732035	0.9712686
Control	2	0.021	Young	0.1628049	1.6290056	2.9480235	0.5208875	8.9309807	4.0630877	0.3242907

Treatment	Experi-ment	Weight	Age	Antho-cyanin	Quer-cetrin	Chlorogenic	Quercetin	Chichoric	Chlorogenic	Chlorogenic
Control	2	0.022	Young	0.0000000	0.7314163	0.1593412	0.2105743	3.2860565	1.1023042	1.8820699
Control	2	0.022	Young	0.1026679	0.5666764	0.2032667	0.1769513	3.6666882	0.8172965	1.5268307
Control	2	0.019	Young	0.2285478	1.7541721	1.8154676	0.4312645	9.7120789	4.5001642	0.4011221
Control	2	0.023	Young	0.0000000	0.4935659	0.1775468	0.1332313	4.2155527	0.8717805	1.0988571
Control	2	0.022	Old	0.4390236	3.4758755	1.5553005	1.1027303	7.2811835	4.7492341	0.2702025
Control	2	0.019	Old	0.5049669	3.7825294	3.5759530	1.1192740	11.1563840	4.7153354	0.3773520
Control	2	0.023	Old	0.0000000	0.5830053	0.1406316	0.2459779	3.5922133	0.9626938	2.7961407
Control	2	0.023	Old	0.9272625	3.1976124	0.7701765	0.7467735	5.8983094	6.3618799	0.2865540
Control	2	0.019	Old	0.9215829	4.4604898	3.6579417	0.9734874	9.5837932	7.6956523	0.0075371
Control	2	0.021	Old	0.3960231	1.9323612	0.3779082	0.6738875	6.0191228	2.1995138	3.7881047
Control	2	0.02	Old	0.9790063	3.7554836	2.6189686	1.2599087	17.3578535	4.4390682	0.2566735
UVA	2	0.025	Young	0.2362865	1.8619668	0.2155224	0.5696206	7.4187642	2.9566960	2.0390349
UVA	2	0.022	Young	0.4640423	2.2570111	0.3523462	0.6760161	7.2807066	3.4462128	1.8199836
UVA	2	0.023	Young	0.1035163	0.9529781	0.2066685	0.3042222	6.2385508	2.0226099	1.0924835
UVA	2	0.025	Young	0.0184902	0.7448919	0.1274056	0.2332533	4.7219666	1.0971465	1.9391150
UVA	2	0.021	Young	0.0789456	1.0598172	0.1255065	0.3461456	4.6551864	0.9916458	1.7754996
UVA	2	0.02	Young	0.0000000	0.6744174	0.2780697	0.2050138	6.0475872	1.1921064	1.0422824
UVA	2	0.024	Young	0.0000000	0.7858700	0.0467220	0.2278508	0.9885712	0.3636604	0.7992036
UVA	2	0.023	Old	0.2179935	2.6302167	0.2511041	0.8352994	8.0385926	1.7753340	3.4860731
UVA	2	0.023	Old	2.2928616	6.2888183	1.7796708	1.3578011	11.8440039	13.1056787	0.1582974
UVA	2	0.022	Old	1.5213673	6.2096398	1.6454683	2.0216216	12.1430680	8.8180521	0.2421953
UVA	2	0.023	Old	2.8793691	8.9659153	1.6185874	1.5757953	10.2859185	10.0791143	0.1566602
UVA	2	0.023	Old	1.6720145	6.7832521	2.2655602	2.1796801	11.7550186	11.7550186	0.3895787
UVA	2	0.02	Old	0.5133042	4.1603960	0.5078008	0.8332258	6.7975977	4.0404005	0.1712475
UVA	2	0.024	Old	0.2733909	4.4427822	0.3531873	0.8790681	4.9625981	4.7371191	0.3390145
UVA/UVB	2	0.024	Young	0.0000000	2.3028375	0.0466361	0.4963550	0.9059964	0.4257403	0.3644759

Treatment	Experi-ment	Weight	Age	Antho-cyanin	Quer-cetrin	Chlorogenic	Quercetin	Chichoric	Chlorogenic	Chlorogenic
UVA/UVB	2	0.024	Young	1.1083315	8.9868364	2.8064537	2.8713605	6.6608171	2.6189110	1.7649168
UVA/UVB	2	0.024	Young	0.7651766	6.3666075	0.1965512	1.6099013	5.6225525	2.6823181	0.4060023
UVA/UVB	2	0.02	Young	1.9366800	6.7477798	0.5604167	1.0597812	10.2500405	5.7134149	0.8813677
UVA/UVB	2	0.022	Young	2.1881311	9.9895116	0.5107514	2.4890249	7.3348387	5.7344962	0.3641660
UVA/UVB	2	0.023	Young	1.6591888	6.9716723	0.6046317	2.0103829	8.7500958	5.7907596	1.2439769
UVA/UVB	2	0.022	Old	0.6780554	11.4676212	0.8679579	1.7649039	5.4820953	3.0868432	0.1302391
UVA/UVB	2	0.023	Old	0.0000000	8.3804968	0.1235762	2.1853069	2.1160821	1.2001319	0.0283922
UVA/UVB	2	0.02	Old	4.8596470	18.4125588	1.2674077	5.1747835	15.7138925	5.1805978	0.2721353
UVA/UVB	2	0.02	Old	5.3067819	21.5785312	1.5960811	5.5131219	12.5931926	10.1933034	0.0905246
UVA/UVB	2	0.022	Old	4.8649695	16.0913545	5.0103789	5.7011495	18.7680522	11.3264810	0.2145017
UVA/UVB	2	0.023	Old	10.2414635	3.3151744	1.8447666	6.6398874	13.9203864	14.4331830	0.0522449



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