Plant Physiology and Biochemistry 93 (2015) 56–65

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

The effects of UV radiation during the vegetative period on antioxidant compounds and postharvest quality of broccoli (*Brassica oleracea* L.)

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ARTICLE INFO

Article history:
Received 30 September 2014
Accepted 23 February 2015
Available online 24 February 2015

Keywords:
Broccoli
Brassica oleracea
UV radiation
MAP
Storage
Quality

ABSTRACT

In this study, the effects of supplementary UV radiation during the vegetative period on antioxidant compounds, antioxidant activity and postharvest quality of broccoli heads during long term storage was studied. The broccolis were grown under three different doses of supplementary UV radiation (2.2, 8.8 and 16.4 kJ/m^2/day) in a soilless system in a glasshouse. Harvested broccoli heads were stored at 0 °C in modified atmosphere packaging for 60 days. The supplementary UV radiation (280–315 nm) during the vegetative period significantly decreased total carotenoid, the chlorophyll a and chlorophyll b content but increased the ascorbic acid, total phenolic and flavonoid contents of broccolis. All supplementary UV treatments slightly reduced the antioxidant activity of the broccolis, however, no remarkable change was observed between 2.2 and 8.8 kJ/m^2 radiation levels. The sinigrin and glucotropaeolin contents of the broccolis were substantially increased by UV treatments. The prolonged storage period resulted in decreased ascorbic acid, total phenolic and flavonoid contents, as well as antioxidant activity. Discoloration of the heads, due to decreased chlorophyll and carotenoid contents, was also observed with prolonged storage duration. Glucosinolates levels showed an increasing tendency till the 45th day of storage, and then their levels started to decline. The weight loss of broccoli heads during storage progressively increased with storage time in all treatments. Total soluble solids, solids content and titratable acidity decreased continuously during storage. Titratable acidity was not affected by UV radiation doses during the storage time whereas soluble solids and solids content (dry matter) were significantly affected by UV doses. Supplementary UV radiation increased the lightness (L^*) and chroma (C^*) values of the broccoli heads. Pre-harvest UV radiation during vegetative period seems to be a promising tool for increasing the beneficial health components of broccolis.

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1. Introduction

During the last few decades, there has been considerable concern over the depletion of the stratospheric ozone layer. The depletion of the ozone layer is closely related to an increase in UV-B radiation on the earth's surface. Although UV-B radiation is only a minor component of total solar radiation, due to its high energy, its potential for causing biological damage is exceptionally high and even small increases could lead to significant biological damage in living plant cells (Zlatev et al., 2012). Plants are vulnerable to increased UV-B radiation because many cellular components, such as nucleic acids, proteins, lipids and quinones can absorb UV-B radiation directly (Jordan, 1996). Thus, the effects of increased UV-B radiation on growth and physiology of different agricultural crops, grown in greenhouse or open-field conditions, have become an important area of study.

The UV wavelength band ranges from 100 to 400 nm and UV radiation is generally divided into three sub-bands UV-A (320–400 nm), UV-B (280–320 nm) and UV-C (200–280 nm) wavelength ranges (Gondor et al., 2014).

Plants are affected by numerous stress factors, and exposure and impact are location, species or even variety specific. UV radiation

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http://dx.doi.org/10.1016/j.plaphy.2015.02.016
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can be regarded as both a stress factor and a regulatory factor. UV can significantly affect plant growth and nutritional quality. Thus, UV-B is an important abiotic environmental factor that can cause many direct and indirect effects on plants (Tsurunaga et al., 2013). For example, UV radiation impacts on the pathways involved in the biosynthesis of the three principal groups of secondary metabolites. These metabolites are phenolics, terpenes, and nitrogen-containing compounds (Cisneros-Zevallos, 2003).

A number of studies have demonstrated that supplemental UV-B radiation can have adverse effects, not only on plant physiological processes (Rathore et al., 2003), but also on photosynthetic CO_2 assimilation, and photosynthetic efficiency (Xiaoqin et al., 2008). UV-B can influence plant processes either through direct damage or alternatively via various regulatory mechanisms, including via the UVR8 photoreceptor response (Potters et al., 2009).

Different plant responses to supplemental UV-B radiation have been established, some are injurious, but there are many recent studies which report that the use of UV-B radiation can be beneficial, especially by increasing levels of health beneficial substances in different plant products (Avena-Bustillos et al., 2012; Du et al., 2014).

Plants may produce secondary products to protect themselves against UV radiation damage. However, these metabolites also play an important role in crop quality and impact on human health. For example, phenolics, flavonoids and anthocyanins are responsible for antioxidant activity in fruits and vegetables (Tsormpatsidis et al., 2008). UV-B can induce a range of specific plant responses, some of which are particularly desirable from a horticultural perspective (Jansen et al., 2008). Park et al. (2007) reported that UV-B can increase the development of color in salad leaves, and control plant disease-tolerance and morphology. For some fruit crops, UV-B exposure has been reported to increase the synthesis of UV-B absorbing compounds of flavonoids and other phenolics (Interdonato et al., 2011). There are even some studies which indicate that postharvest UV-B radiation increases total soluble phenolics and flavonoids content and antioxidants in fruit and vegetables (Du et al., 2014). The UV-B induced increase in antioxidative defenses is further demonstrated by increases in both the reduction state and pool-size for antioxidants such as ascorbate, glutathione, xanthophylls, and tocopherol (Jansen et al., 2008).

In recent years, the production of cabbages and other brassicas has increased, due to their beneficial health effects. Generally, consumption of *Brassica ssp*. vegetables, especially broccoli, have been claimed to exert protective effects against cancer due to their rich contents of glucosinolates (including glucobrassicinand glucoraphanin), flavonoids (quercetin, kaempferol etc.), vitamins (C and E) and other mineral nutrients (Jeffery and Araya, 2009). Similarly, epidemiological processes indicate that a frequent intake of cruciferous vegetables, such as broccoli, can highly diminish the risk of bladder cancer (Liu and Lv, 2013).

Since broccoli is a highly perishable vegetable, the preservation of its nutrient content and antioxidant activity during the postharvest period is extremely important (Serrano et al., 2006). Broccoli is susceptible to yellowing after harvest, which negatively affects its nutritional and commercial value as well. Generally, the "head quality" of broccoli markedly decreases when flowering heads are kept at room temperature for 3–5 days after harvest. So, it is essential that this fast postharvest yellowing is avoided during handling and storage to protect the nutritional quality, and to prolong the postharvest life of this perishable crop. To maintain the postharvest quality of broccolis, it is crucial to store heads at low temperatures as soon as possible after harvesting. Low temperature (0–4 °C) and high relative humidity (90–95%) are required to maintain the postharvest quality of broccoli heads. Modified atmosphere packaging (MAP), in combination with low temperature, is simple, beneficial, and economical and also the most effective method to delay postharvest yellowing and deterioration and to maintain the visual and postharvest quality of broccolis (Serrano et al., 2006).

There are several studies on the effects of UV-B radiation on the postharvest quality, and phytochemicals and antioxidant compound content in harvested broccoli (Aiamla-or et al., 2010; Rybarczyk-Plonska et al., 2014). However, there are only a few experiments which have involved analyzing the effects of supplemental UV-B radiation, during the broccoli vegetative growth and development period, on the postharvest quality parameters.

The objective of this present study is to investigate the effect of different supplementary UV radiation doses, given during the vegetative period of glasshouse grown broccoli, on postharvest characteristics, physiological and biochemical parameters, and antioxidant compounds. The long term aim is to provide information that leads to a longer storage life and improved phytochemical properties for this short-lived nutritious vegetable.

2. Materials and methods

2.1. Plant material and experiment site

Broccoli seedlings (*Brassica oleracea* L. italica var. Naxos F1) were grown in a soilless culture in a growing mixture with a peat/perlite (1:1 v/V) in a glasshouse in the experimental unit of Akdeniz University in Antalya, Turkey (36°53′ N; 30°39' E, altitude 39 m). The annual number of sunny days at the experimental site is approximately 300 days. Inside the glasshouse, PAR radiation intensity was about 34% of the outdoors levels around solar noon, when the UV treatments were applied. No UV-B absorbing film was used in the experimental glasshouse so a small amount of UV-C light, emitted by the UV-tubes, would have reached plants in the glasshouse.

2.2. UV-B radiation exposures

Broccoli seedlings having 3 true leaves (13.9 cm height) were obtained from a commercial seedling company and they were planted in the glasshouse on January 7th 2014. After planting, the glasshouse was separated into four groups for different UV radiation treatments. The first group of plants was radiated with 2.2 kJ/m^2/day, the second and third group of broccoli plants were radiated with 8.8 and 16.4 kJ/m^2/day, respectively. The fourth and last group of plants was the control group and these broccoli plants received no supplemental UV radiation during the entire vegetation period. Since broccoli seedlings are rather sensitive to UV radiation, the first radiation exposure was applied 15 days after planting. Philips narrow band (TL F72T12 100W/01 UV-B) UV lamps were used for the radiation exposures (280–315 nm). The different UV radiation doses were obtained by altering the duration of the exposure at the fixed distance of 15 cm above the upper parts of plants. Prior to use, the UV lamps were allowed to stabilize by turning them on at least 15 min beforehand. From fifteen days after planting till harvesting, UV exposures were applied to the broccoli on a daily basis. These radiation durations were 27 min (for 2.2 kJ/m^2), 64 min (8.8 kJ/m^2) and 120 min (16.4 kJ/m^2).

2.3. Storage of broccoli heads

Broccoli grown in a soilless culture in a glasshouse was harvested 91 days after planting, at the commercial maturity stage (average head weight 450–500 g; total soluble solids (TSS) 7.76%; total titratable acidity (TTA) 0.17%) on May 8th 2014. The harvested broccoli heads were immediately transported to the postharvest laboratory and cold storage unit of the Department of Horticulture,

Akdeniz University in Antalya, Turkey for pre-cooling and storage.

Broccoli heads free of visual defects and with a diameter ranging between 13 and 14 cm were chosen, and the inner branches (with floret stalks of approximately 2 cm) were then cut from these heads for experimentation. Selected uniform broccoli heads were placed in plastic boxes and packed in modified atmosphere packaging (MAP) (MAP: made of Xtend® film XF.A12 (Cod: 815-PG3, Patent No: 6190710, StePac Co., Antalya, Turkey). After MAP, the broccoli heads obtained from the different UV-exposure treatments were stored at a temperature of 0 °C with 90–95% relative humidity for 60 days. During the storage, various quality analyses were performed on the heads taken from different storage conditions at fifteen day intervals.

2.4. Weight loss

At harvest, individually numbered heads were weighed, and at the end of each storage period 18 heads per treatment with three replicates were re-weighed. Weight loss was expressed as percent loss from the initial weight.

2.5. Head color

The color of heads (three measurements of each individual head) was measured on 18 heads from each UV-exposure replicate using a chromameter (CR 200, Minolta, Ramsey, NJ, USA). CIE L^*, a^*, and b^* values of broccoli heads were recorded. In the CIELAB color chart, negative a^* values indicate green and positive a^* values indicate red color. Higher positive b^* values indicate a more yellow skin color and negative b* blue color. These values were then used to calculate a Hue angle, where 0° = red-purple; 90° = yellow; 180° = bluish green; and 270° = blue and Chroma, which indicates the intensity or color saturation.

2.6. Total solids, soluble solids and titratable acidity

Total solids content of the samples were determined by drying the pureed broccoli (~5–6 g) in an oven at 70 °C until insignificant consecutive weight changes were observed. The results were given as % dry matter (DM).

Broccoli heads were squeezed with cheesecloth and the juice obtained was analyzed for total titratable acidity and total soluble solids. Total titratable acidity (TTA) was determined by titrating 2 ml of broccoli juice in 38 ml of distilled water with 0.1 N NaOH to an end point of pH 8.1. TTA was expressed as percent of citric acid equivalents. Total soluble solids (TSS) were measured by a digital refractometer (Model Number REF121, Atago, China) and expressed as %.

2.7. Decay incidence

Decay incidence of broccoli heads was visually evaluated during the experiment. Any broccoli heads with visible mold growth were considered decayed. Decay was expressed as a percentage of total heads.

2.8. Head appearance

During storage, broccoli heads were evaluated by panelists for appearance. A panel of ten trained evaluators used a scale of 1–5, where 1 indicates extremely bad appearance and 5, extremely good appearance 1 = very bad; 2 = bad; 3 = medium (limit of marketability); 4 = good; 5 = excellent. Scores of 3 or above were considered acceptable for commercial purposes.

2.9. Ascorbic acid content

Spectrophotometric determination of the ascorbic acid content of broccoli samples was performed. For this purpose, a fresh broccoli sample was mixed with metaphosphoric acid solution (6%) in the ratio 1:1 (w/w) and pureed by a hand blender. Five ml of filtered broccoli extract was mixed with 5 ml of acetate buffer. Subsequently, 1 ml of dye solution (2,6-dichlorophenol indophenol) and 10 ml xylene were added to this mixture. The blank sample was prepared in the same way and 5 ml of metaphosphoric acid solution was used instead of broccoli extract. These mixtures were then centrifuged (×6000 *g*) at 25 °C for 2 min. The absorbance of the upper xylene layer was measured at 515 nm. The calibration curve of authentic L-ascorbic acid was used to determine the ascorbic acid content of the samples. The results were expressed as g ascorbic acid/100 g dry matter.

2.10. Total phenolics, total flavonoids, and antioxidant activity

Fresh broccoli juice was obtained by using a juicer and mixed with methanol in the ratio 1:1 (v/v). This mixture was then centrifuged at 6.000 *g* and 4 °C for 5 min. The supernatant was used for the following analyses.

For total phenolics content, first 0.5 ml of the sample extract was treated with 2.5 ml of 0.2 N Folin–Ciocalteu's phenol reagent and 2 ml of Na_2CO_3 (75 g/l). The mixture was incubated at 50 °C for 5 min and then immediately cooled to room temperature. The absorbance of the final solution was measured by spectrophotometer (UV–Vis 160A, Shimadzu, Japan) at 760 nm, and compared with respect to the blank solution (0.5 ml of 80% methanol solution in place of sample extract). Results were expressed as a gallic acid equivalent (mg of GAE/g dry matter).

For total flavonoids content, volumes of 2.5 ml distilled water and 150 μL of 5% $NaNO_2$ solution were added to 0.5 ml of the sample extract. The mixtures were vortexed and allowed to stand for 5 min at room temperature. After that, 300 μL of 10% $AlCl_3$ was added to the solution and the mixture was allowed to stand for 5 min again. A further 1 ml of 1 M NaOH was added and the final volume was made up to 5 ml with distilled water. Sample absorbance at 510 nm was measured by using a spectrophotometer (Shimadzu UV–Vis 160A, Japan) against a prepared blank solution 80% aqueous methanol). The results were expressed as (+)-catechin equivalent (mg of CE/g dry matter).

The antioxidant activity of the sample was analyzed by using the DPPH method. A diluted sample of extract (100 μL) (prepared at 5 different concentrations that showed 10%–90% inhibition for DPPH radical) was added to 4 ml of freshly prepared DPPH (2.2-diphenyl-1-picryhydrazyl radical) solution (6×10^{-5} M in MeOH). The mixtures were shaken and kept in the dark at room temperature for 30 min. Absorbance at 516 nm was measured using a spectrophotometer (UV–Vis 160A) and compared with respect to a control solution (aq. 80% MeOH v/v instead of extract in DPPH solution). The antioxidant activity of the samples was expressed as a percent inhibition of the DPPH radical and calculated by the following equation;

$$I(\%) = \left[\left(A_{C(0)} - A_{S(t)}\right) \Big/ A_{C(0)}\right] \times 100 \quad t = 30 \text{ min}$$

where I is the inhibition percentage, and Ac and A_A are the absorbance values of the control and test samples, respectively. The sample extract concentration providing 50% inhibition (IC50) of the DPPH radical was calculated from the plot of concentration versus percent inhibition.

2.11. Determination of chlorophyll a, chlorophyll b and carotenoid content

The total chlorophyll content of broccoli samples was determined by the method of Lichtenthaler and Wellburn (1983). For this purpose, a 0.25 g fresh sample was homogenized and the volume was made up to 25 ml with aceton (80%). Absorbances at 663 nm and 645 nm were taken by using a spectrophotometer (Shimadzu UV–Vis 160A, Japan). The following equations were used to calculate *chlorophyll a* and *chlorophyll b*;

$$Chlorophyll\ a(mg/g) = (12.7 \times A_{663}) - (2.69 \times A_{645}) \times V/W \times 1000$$

$$Chlorophyll\ b(mg/g) = (22.91 \times A_{645}) - (2.69 \times A_{663}) \times V/W \times 1000$$

where V is the volume of the extract and W is the weight of sample.

For total carotenoids content, an amount of 30 g of homogeneous puree material was mixed with 5 g of HyfloSupercel and 75 ml of methanol (%70). The mixture was then filtered through What man filter paper (No.2). The residue of the mixture was substantially extracted two times by using 75 ml of acetone-petroleum ether in the ratio of 1:1 (w/w). Approximately 225 ml of extract was placed in a separating funnel, containing 25 ml KOH (%10). The separating funnel was shaken and left for/layers separation for 45 min. After this time, 75 ml petroleum ether and 100 ml NaCl (%20) were added and the separating funnel was shaken again. The hypophasic layer was removed and the epiphasic layer was splashed with water three times. Finally, the epiphasic layer was filtered from a filter paper by using anhydrous Na_2SO_4. The clear extract obtained was transferred to a 250 ml volumetric flask and the volume was made up to line with petroleum ether. The absorbance spectrum of petroleum ether extracts was determined between 350 and 750 nm. The maximum absorbance for β-carotene was recorded at 438 nm and the result was expressed as mg β-carotene/100 g dry matter. The following equation was used for calculation of total carotenoids content; Total carotenoid = (A). $8.33/\epsilon \times 1000$ ($\epsilon = 2500$ for β-carotene).

2.12. Glucosinolate content

Glucosinolates *(sinigrin and glucotropaeolin)* were quantified using a method adapted from Rosecler et al. (2008) with some modification. 125 mg of a freeze dried sample was transferred to centrifuge tubes, and 1 ml of 70% methanol + 1 ml 0.1% trifluoroacetic acid (TFA) were added to each tube. The homogenates were shaken for 20 min at 70 °C and left to stand until they reached room temperature. This sample was centrifuged at 13,000 *g* and the supernatant was then filtered through a membrane (0.45 μm) into injection vials. The extracts were analyzed in HPLC. The chromatographic separation was performed on a solvent delivery system (20AD, Shimadzu, Japan) coupled with an autosampler (SIL-20A Prominence, Shimadzu, Japan), column (LiChroCART®250-4250 mm × 4 mm 5 μm Nucleosil®100-5C 18) and a guard column LiChroCART® 4-4 Nucleosil® 5C 18), maintained at 30 °C in a column oven (CTO-20AC, Shimadzu, Japan).

Individual peaks (Fig. 1) were detected at 228 nm by an SPD-M20A Diode Array Detector (Shimadzu, Japan) which is controlled by LC solution software. Separation was performed using the following solvents: A, 0.1% (v/v) TFA in dionized water; and B, 0.1% (v/v) TFA in methanol. The elution gradient was as follows: 100% A for 10 min; 80% A and 20% B from 10 to 15 min; 50% A and 50% B from 15 to 25 min; 100% B from 25 to 35 min. The flow rate was 0.8 ml/min. Individual peaks were identified by comparison of the retention time and spectrum pattern of their authentic standards. They were also validated by spiking. Quantification was performed by the plotting of five different external standard concentrations versus peak areas.

2.13. Statistical analysis

Groups of three replicates of each 18 heads per UV treatment for the storage periods were established. The data were analyzed using the Statistical Analysis System software program, version 9.0 (SAS Inst., Cary, N.C. U.S.A.) and treatments means were statistically compared using the Duncan's multiple range test.

3. Results and discussion

3.1. Weight loss

Water loss is a common storage problem of broccoli heads which affects commercial marketing of this crop, just like that of many other fresh fruit and vegetables. There were significant effects of UV radiation dose, storage time, and UV radiation dose × storage time interaction on the weight loss after 60 days of cold storage ($P \leq 0.05$). The weight loss of broccoli heads progressively increased with storage time in all treatments (Table 1).

Our results show that, the UV radiation strongly inhibited weight loss of broccoli during storage compared to the control broccoli. The control broccoli lost 10.00% of their weight during the 60-day storage period. During the same storage period, the broccoli exposed to 2.2, 8.8 or 16.4 kJ/m^2 UV radiation lost 6.70%, 6.48% and 5.89% of their initial weights, respectively (Table 1).

Consistent with our results, other studies have shown that UV-B radiation reduced stomata opening in broccoli and that this caused a decline in weight loss (Kaewsuksaeng et al., 2012). Our results also showed that cooling to 0 °C and the modified atmosphere created by MAP greatly reduced the weight loss of broccoli heads, as was also reported in earlier study (Serrano et al., 2006).

3.2. Decay incidence

In our study, decay incidence increased with storage time. In all treatments, head decay was not observed during the first 15 days in any treatments, or even 30 days in control heads. After 60 days of storage, decay percentages in the control and 16.4 kJ/m^2 UV treated broccoli were 1.67% (Table 1). In heads exposed to 2.2 and 8.8 kJ/m^2 the incidence of decay was, respectively, 2.0 and 3.0% at the end of the same 60 days storage.

Demkura and Ballare (2012) reported that UV-B treated plants are more resistant to the attack of leaf pathogens than untreated control plants. In our experiment there were no statistical differences between UV radiation doses on decay incidence of broccoli heads. However, there were statistically significant differences between storage periods. Similar to our results, Martinez-Hernandez et al. (2013) reported that throughout the storage period yeast and mold levels on broccoli samples increased slightly.

3.3. Head color

The most important factor for marketing of broccoli is retention of the green color of heads. This is a key determinant of consumer preference. Retention of green color and visual quality occurred in all broccoli heads during MAP at 0 °C.

The L^* value is a measure of the lightness of the sample, the C^* value describes its brightness while the h^o value represents true color. The effects of different UV radiation doses, storage time and

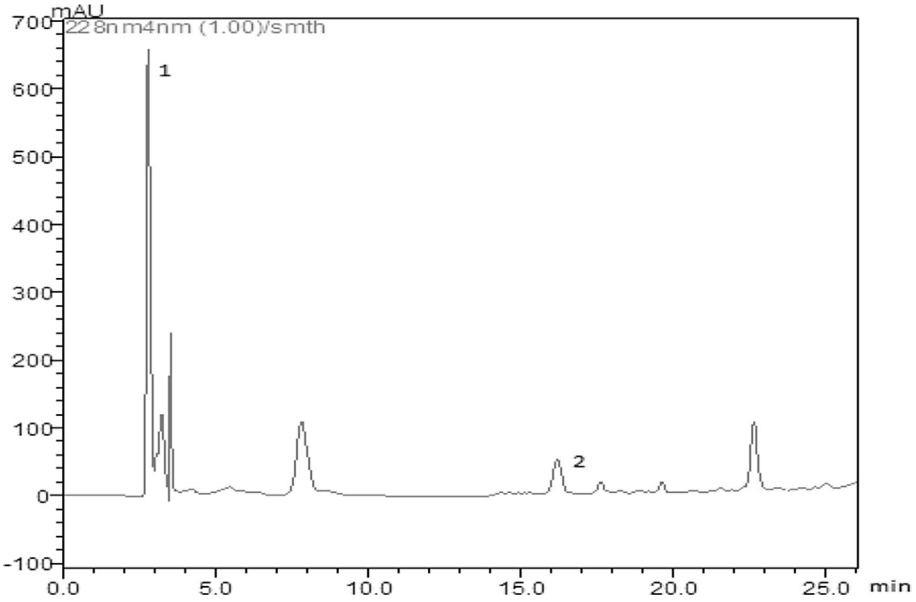

Fig. 1. Chromatogram of the sample extract (1: Sinigrin, 2: Glucotropaeolin).

Table 1
Weight loss, decay incidence, L^*, C^*, h^o values and head appearance of 'Naxos F_1' broccolis during storage at 0 °C.

Testing index	Treatments	Storage duration (days)					
		Day 0	Day 15	Day 30	Day 45	Day 60	Overall
Weight loss	Control	–	2.61 fgh	5.18 b-f	7.65 ab	10.00 a[a]	6.36 A[b]
(%)	2.2 kj	–	1.75 gh	2.92 e-h	4.49 c-g	6.70 bc	3.96 B
	8.8 kj	–	1.84 gh	3.73 d-h	5.43 b-e	6.48 bcd	4.37 B
	16.4 kj	–	1.34 h	3.04 e-h	4.88 b-f	5.89 bcd	3.79 B
	Overall		1.88 d	3.72 c	5.61 b	7.27 a	
Decay incidence (%)	Control	0.00	0.00	0.00	0.33	1.67*	0.40[c]
	2.2 kj	0.00	0.00	1.33	2.00	3.00	1.27
	8.8 kj	0.00	0.00	0.67	1.33	2.00	0.80
	16.4 kj	0.00	0.00	0.00	1.00	1.67	0.53
	Overall	0.00 b	0.00 b	0.50 b	1.17 ab	2.08 a	
L^*	Control	37.25 ı	38.28 h	39.07 fg	39.10 fg	39.25 f	38.59 D
	2.2 kj	38.82 g	39.23 f	39.82 e	40.07 e	40.12 e	39.61C
	8.8 kj	40.11 e	41.10 d	41.72 c	41.75 c	41.79 c	41.30 B
	16.4 kj	42.62 b	42.70 ab	42.84 ab	42.90 ab	43.01 a	42.81 A
	Overall	39.70 D	40.33C	40.87 B	40.95 AB	41.04 A	
C^*	Control	20.94 m	21.06 lm	21.09 l	21.14 kl	21.25 k	21.10 D
	2.2 kj	23.65 j	23.91 ı	24.08 h	24.19 gh	24.26 g	24.02C
	8.8 kj	24.63 f	24.93 de	24.90 e	24.98 de	25.05 d	24.90 B
	16.4 kj	25.45 c	25.75 b	25.77 b	25.88 ab	25.95 a	25.76 A
	Overall	23.67 D	23.91C	23.96C	24.05 B	24.13 A	
h^o	Control	121.12 bcd	122.86 a	121.93 bc	122.00 b	121.93 bc	121.97 A
	2.2 kj	120.20 def	120.34 def	120.16 ef	120.03 f	119.98 f	120.14C
	8.8 kj	121.01 de	120.81 def	121.06 cde	121.12 bcd	121.15 bcd	121.03 B
	16.4 kj	120.54 def	120.70 def	120.90 def	120.97 de	121.00 de	120.82 B
	Overall	120.72 B	121.18 A	121.01 AB	121.03 AB	121.02 AB	
Head	Control	5.00 a	5.00 a	5.00 a	5.00 a	4.67 ab	4.93[c]
appearance[d]	2.2 kj	5.00 a	5.00 a	4.67 ab	4.33 ab	4.33 ab	4.67
	8.8 kj	5.00 a	5.00 a	4.67 ab	4.33 ab	4.00 b	4.60
	16.4 kj	5.00 a	5.00 a	4.67 ab	4.33 ab	4.00 b	4.60
	Overall	5.00 a	5.00 a	4.75 ab	4.50 bc	4.25 c	

[a] Means in the same row with different letters are significantly different at $P \leq 0.05$ by Duncan's multiple range test.
[b] Means in the same column with different letters are significantly different at $P \leq 0.05$ by Duncan's multiple range test.
[c] Means in the same column or same row is not significantly different at $P \leq 0.05$ by Duncan's multiple range test.
[d] The head appearance was conducted on the base of a 5 point scale, 1 = very bad; 2 = bad; 3 = medium (limit of marketability); 4 = good; 5 = excellent.

their interaction on the L^*, C^* and h^o values of broccoli heads were found to be significant ($P \leq 0.05$).

The L* value of the heads slightly increased during storage (Table 1). After 60 days storage, the highest L* value of the broccoli was for heads exposed to 16.4 kJ/m^2 UV (43.01). The lowest L* value was for control heads (39.25). Similar to our study, Ranjbarfordoei et al. (2011) reported that UV-B radiation increased L* value in broccoli, and this was hypothesized to be due to chlorophyll degradation.

The C^* values generally increased during the storage, similar to L

values. The highest C* value after 60 days of storage was measured in heads exposed to 16.4 kJ/m^2 UV radiation (25.95). The lowest C* value was found in control heads (21.25) (Table 1).

The trend in h^o values of broccoli heads was given in Table 1. The highest h^o value was found in control group heads (121.93) and the lowest value in heads exposed to 2.2 kJ/m^2 UV (119.98). There was no significant difference in h^o values of the samples, treated with 8.8 or 6.4 kJ/m^2 UV radiation (overall values).

Similar to our results, UV-B radiation has been found to show delayed floret yellowing and chlorophyll degradation in broccoli heads (Aiamla-or et al., 2010).

3.4. Head appearance

Broccoli is a highly perishable product and its postharvest life and visual quality greatly depend on storage conditions, such as temperature, atmosphere composition and relative humidity. In our study high levels of supplemental UV radiation during the vegetative period negatively affected head appearance and resulted in uneven head formation (Fig. 2). Visual appearance decreased with storage time in all treatments (Table 1). The head appearance did not change in control heads during the first 45 days of storage. After 45 days of storage, head appearance had decreased in control heads and at the end of 60 days head appearance of broccolis was scored as 4.67 (Table 1). Head appearance of UV-exposed samples started to change after 15 days. Similar to control heads, head appearance of 8.8 and 16.4 kJ/m^2 UV treated heads decreased with storage and was scored as 4.00 following 60 days storage, which means good and still marketable. Similar decreases in head appearance were also found in broccoli heads by Jia et al. (2009) during cold storage under MAP conditions.

3.5. Total solids, soluble solids, and titratable acidity

UV treatments and storage periods significantly ($P < 0.05$) affected the total solids (TS), total soluble solids (TSS) and total titratable acidity (TTA) content of broccoli heads (Table 2). In our study, TS, TSS and TTA decreased as the storage period progressed in all treatments. These decreases were slower in the control heads than in heads of UV treated broccolis. TS content of the control heads was 12.51% at harvest and decreased to 9.79% after 60 days of storage. A similar decreasing tendency during storage was observed in UV treated broccolis as well. The lowest TS content was observed in heads grown under 16.4 kJ/m^2 UV, this was 6.79% after a 60 day storage period.

UV treatments and storage periods significantly ($P < 0.05$) affected the total soluble (TSS) content of broccolis (Table 2). In our study, TSS decreased as the storage period progressed in all treatments during storage. These decreases were slower in control heads than those of UV treated broccolis. TSS content of the control heads was 9.00% at harvest and decreased to 8.10% after 60 days of storage. A similar decreasing tendency during storage was observed in UV treated broccolis as well. The lowest TSS content was observed in heads grown under 16.4 kJ/m^2 UV, this was 6.83% after a 60 day storage period.

The effects of UV radiation on storage time and interaction of UV radiation × storage time on the total titratable acidity (TTA) were significant ($P \leq 0.05$) (Table 2).

At harvest, the TTA content of the control broccolis was 0.20%. TTA content decreased continuously in all treatments during the

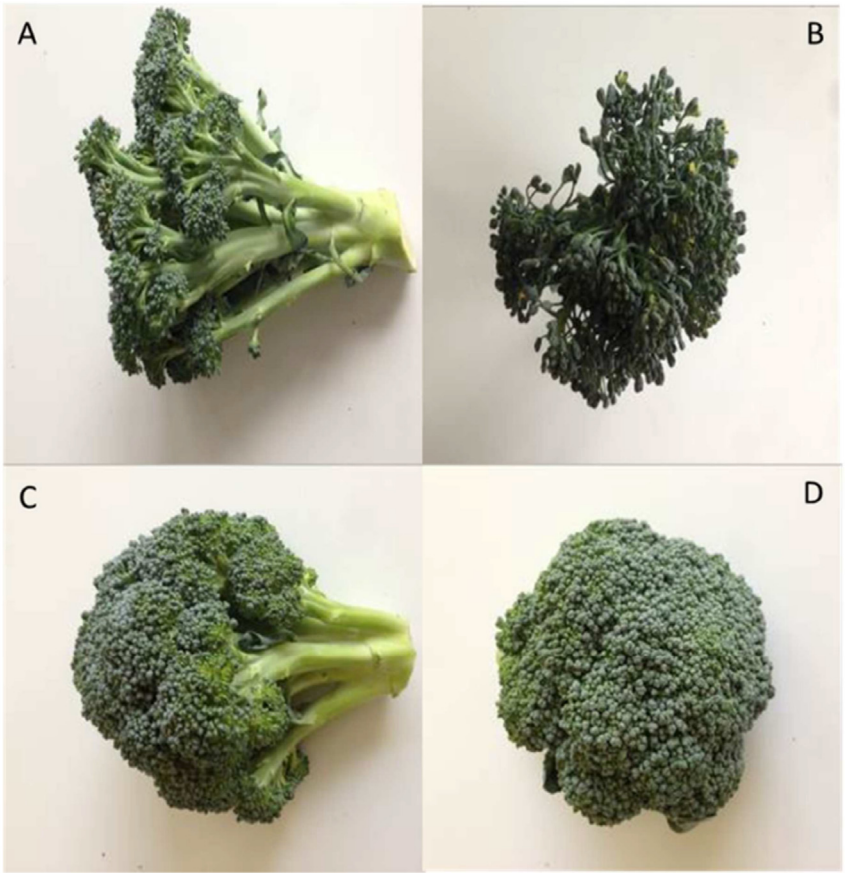

Fig. 2. Uneven head formation at 16.4 kJ/m^2 radiated broccolis (A–B) and compact head formation (C–D) (Heads were harvested after 91 days from planting).

Table 2
Total solids (dry matter), soluble solids, titratable acidity, chlorophyll a and chlorophyll b contents of 'Naxos F_1' broccolis during storage at 0 °C.

Testing index	Treatments	Storage duration (days)					
		Day 0	Day 15	Day 30	Day 45	Day 60	Overall
Total solid (%)	Control	12.51 a	9.21 c	8.84 cd	8.97 cd	9.79 de[a]	9.86 A[b]
	2.2 kj	10.76 b	8.28 de	8.22 fe	8.09 f	8.03 fg	8.67 B
	8.8 kj	9.33 c	6.77 hg	7.33 h	7.36 h	7.57 hı	7.67C
	16.4 kj	8.16 fe	7.04 h	7.12 jı	7.00 h	6.79 jk	7.22 D
	Overall	10.19 A	7.83 B	7.88 B	7.85 B	8.04 B	
TSS[c]	Control	9.00 a	8.23 b	7.33 de	8.00 b	8.10 b	8.13 A
(%)	2.2 kj	7.93 bc	8.03 b	8.17 b	7.00 efg	7.33 de	7.69 B
	8.8 kj	7.80 bc	7.53 cd	6.93 efg	7.00 efg	7.20 def	7.29C
	16.4 kj	7.53 cd	6.67 g	6.80 fg	6.87 efg	6.83 fg	6.94 D
	Overall	8.07 a	7.62 b	7.31 c	7.22 c	7.37 c	
TTA[d]	Control	0.20 a	0.16 bc	0.16 bc	0.15 b-e	0.14 de	0.16 A
(% citric acid)	2.2 kj	0.16 bc	0.15 b-e	0.15 b-e	0.14 de	0.11 h	0.14 B
	8.8 kj	0.16 bc	0.14 de	0.14 de	0.14 de	0.12 fgh	0.14 B
	16.4 kj	0.16 b	0.15 b-e	0.15 b-e	0.14 de	0.11 h	0.14 B
	Overall	0.17 a	0.15 b	0.15 b	0.14 c	0.12 d	
Chlorophyll a	Control	9.96 a	9.70 b	9.56 c	9.39 d	9.35 d	9.59 A
(mg/g)	2.2 kj	7.49 e	7.32 f	7.22 g	7.03 h	6.90 ı	7.19 B
	8.8 kj	3.79 j	3.56 k	3.41 l	3.23 m	3.11 n	3.42 C
	16.4 kj	3.25 m	3.03°	2.93 p	2.77 q	2.60 r	2.92 D
	Overall	6.12 A	5.90 B	5.78C	5.60 D	5.49 E	
Chlorophyll b	Control	7.81 e	8.15 d	8.25 c	8.50 b	8.62 a	8.27 A
(mg/g)	2.2 kj	4.88 j	5.02 ı	5.26 h	5.55 g	5.70 f	5.28 B
	8.8 kj	2.79 p	2.88°	3.03 n	3.26 l	3.36 k	3.06C
	16.4 kj	2.19 s	2.44 r	2.68 q	2.95°	3.13 m	2.68 D
	Overall	4.42 E	4.62 D	4.81C	5.06 B	5.20 A	

[a] Means in the same row with different letters are significantly different at $P \leq 0.05$ by Duncan's multiple range test.
[b] Means in the same column with different letters are significantly different at $P \leq 0.05$ by Duncan's multiple range test.
[c] TSS: total soluble solids.
[d] TTA: total titratable acidity.

storage and it decreased to 0.14% at the end of the 60th day of storage. Although there were statistically significant differences between UV radiation doses and the control, there were no statistically significant differences between the UV-B radiation doses (Table 2). Our findings are in agreement with previous studies on TS, TSS and TTA in broccoli (Lima et al., 2013).

Echeverria and Valich (1989) reported that the decrease in TTA at the end of storage might be due to the metabolic changes in fruits and vegetables as a result of the use of organic acids in the respiratory process. The presence of metabolic activity may affect solid soluble content and soluble solids content may be used in the respiration process (Martinez-Hernandez et al., 2013). Upon ripening, ethylene production causes changes in fruit sugar content and organic acid metabolism (Guillen et al., 2006).

3.6. Chlorophyll a, chlorophyll b and carotenoid content

Both UV treatments and storage period showed significant effects on the chlorophyll content of the samples (Table 2). Chlorophyll a contents of the samples were considerably decreased following prolonged storage and in samples exposed to higher doses of UV. Treatment with 16.4 kJ/m^2 UV caused about a 68–70% loss in chlorophyll a and b contents of the samples.

Chlorophyll a content of broccoli samples decreased with the increased dose of UV and storage time (Table 2). At harvest time, chlorophyll a content for control samples was 9.96 mg/g, but this value decreased to 9.35 mg/g after 60 days of storage.

Chlorophyll b content of broccoli samples increased with storage duration (Table 2). The initial chlorophyll b content of the broccoli exposed to 16.4 kJ/m^2 UV was at harvest 2.19 mg/g and this value increased to 2.95 mg/g after 60 days storage.

Postharvest senescence in broccoli during storage caused a yellowish color due to the loss of green color which is due to the degradation of chlorophyll (Yamauchi and Watada, 1998). A significant reduction in chlorophyll a and b contents in response to UV-B exposure was also recorded in previous studies (Ravindran et al., 2010; Shaukat et al., 2013).

As with the chlorophyll a content, the mean carotenoid content of broccoli samples decreased with increased dose of UV and storage time (Table 2). The overall carotenoid content for control samples was 4.19 mg/100 g DM and this value decreased more than 40% (to 2.47 mg/100 g DM when plants were grown under high levels of (16.4 kJ/m^2) UV. At the beginning of the storage period, the mean carotenoid content of heads raised under 16.4 kJ/m^2 UV was 2.64 mg/100 g DM and this value decreased to 2.24 mg/100 g DM after 60 days of storage.

Our results on degradation of chlorophyll a and carotenoids by UV treatment were found to be in agreement with the results of previous work on true indigo (Ravindran et al., 2010). These authors stated that since the carotenoids are involved in the light harvesting and protection of chlorophyll from photo-oxidative destruction, any reduction in carotenoid could have serious consequences on chlorophyll pigments.

Similar to our findings, it has been reported that UV (254, 302 and 365 nm) radiation resulted in reduction in the amount of chlorophyll a as opposed to chlorophyll b and might point to more selective destruction of chlorophyll a biosynthesis or degradation of precursors (Marwood and Greenberg, 1996). However, in some studies the UV exposure stimulated the biosynthesis of UV-absorbing compounds and carotenoids, both of which perform a photoprotective function. The carotenoids are implicated in the direct protection of the photosystems against UV radiation (Middleton and Teramura, 1993).

3.7. Total phenolics, total flavonoids, and antioxidant activity

Total phenolic and flavonoid content of broccoli samples were significantly ($P < 0.05$) affected by UV-B treatments and storage

time (Table 3). At harvest, he initial value for total phenolic content of the control samples was 2.17 mg GAE/g DM This value decreased to 1.91 mg GAE/g DM after 60 days storage.

As with the total phenolic content, the total flavonoid content of broccoli samples increased with UV dose, but decreased with prolonged storage (Table 3). The initial total flavonoid content for heads raised under 16.4 kJ/m^2 UV was 0.27 mg CE/g DM. This value decreased to 0.24 mg CE/g DM after 60 days of storage. UV treatment slightly increased total phenolic and flavonoid contents of the samples, this increase was clearest under high UV levels.

Consistent with our results, Ravindran et al. (2010) observed increased concentrations of flavonoids and anthocyanin in *Indigofera tinctoria* seedling following UV-B treatment. Similary, Scattino et al. (2014) reported that UV-B radiation after harvest is an effective tool to modulate the concentration of health-promoting compounds in peach and nectarine fruits and it is able to induce modifications at gene expression level.

Several studies have demonstrated that UV-B can induce a general stress response and as well as specific, UV-regulated physiological and photomorphogenic responses. Shaukat et al. (2013) reported a four-fold increase in the flavonoid content of black gram, exposed to UV-B for 40 min, when compared with the controls. They also found that the rate of accumulation of total soluble phenols was related to UV-B exposure time. Synthesis of phenolic substances such as anthocyanin and flavonoids was also observed in UV-B treated *Arabidopsis thaliana* (L.) seedlings. A role for flavonoids in UV protection is also supported by isolation of *Arabidopsis* mutant which is tolerant of extremely high UV-B levels (Bieza and Lois, 2001). Total phenolic and flavonoid contents of the broccoli samples decreased substantially during increased storage period due to degradation reactions.

The IC_{50} values of the samples ranged between 0.32 and 0.44 mg DM/mg DPPH at the beginning of storage (Table 3). UV treatment slightly reduced (~10% reduction) antioxidant activity of the samples, however, no major difference was observed between heads raised under 2.2 kJ or 8.8 kJ/m^2 UV. Growth under 16.4 kJ/m^2 UV radiation caused a 5% decrease in the antioxidant activity. This slight reduction of antioxidant activity can be associated with the reactive oxygen species (ROS) in the samples due to stress caused by UV radiation. Although the amount of antioxidant compounds, such as phenolics and flavonoids, increased following UV treatment, the ROS produced by UV radiation might nullify their positive influence on the antioxidant activity. Zlatev et al. (2012) have stated that UV-B radiation produces reactive oxygen species which cause oxidative damage in plant cells. The higher antioxidant activity, and thereby lower IC50 value, was estimated for the initial samples just after harvest. Although the antioxidant activity of the samples decreased about 30% in 15 days of storage, no further change was observed between 15 and 60 days of storage period.

3.8. Ascorbic acid

The initial ascorbic acid value of the control samples was 0.33 g/100 g DM at the beginning of storage (Table 3). In broccoli heads raised under 2.2 kJ/m^2 UV, this value was 0.55 g/100 g DM, which indicates an increase of more than 60%. A more than two-fold increase in the ascorbic acid content was observed in heads from the 16.4 kJ/m^2 UV treatment. As expected, prolonged storage negatively affected the ascorbic acid values, which decreased from an average of 0.55 g/100 g DM to the value of 0.37 g/100 g DM after 60 days of storage.

Castagna et al. (2013) stated that postharvest UV-B radiation increased the concentration of ascorbic acid content in the flesh and peel of tomatoes. Similarly, Hagen et al. (2007) found that the content of ascorbic acid in the peel of apples increased after UV-B treatments. Our results are in agreement with the findings of these researchers. In contrast, Liu et al. (2011) found that the content of ascorbic acid in tomatoes decreased after postharvest UV-B treatments.

Zlatev et al. (2012) found a UV-B induced increase in ascorbic

Table 3
Total phenolics, total flavonoids, total carotenoids, ascorbic acid and antioxidant activity of 'Naxos F_1' broccolis during storage at 0 °C.

Testing index	Treatments	Storage duration (days)					
		Day 0	Day 15	Day 30	Day 45	Day 60	Overall
Total phenolics	Control	2.17 h	2.24 gh	2.17 h	2.13 h	1.91 h[a]	2.12 D[b]
(mg GAE/g DM)	2.2 kj	2.47 f	2.47 f	2.40 fg	2.37 fg	2.37 fg	2.42C
	8.8 kj	3.15 d	3.42 c	3.02 de	3.01 de	2.91 e	3.10 B
	16.4 kj	3.79 a	3.77 ab	3.68 ab	3.63 ab	3.60 b	3.69 A
	Overall	2.90 AB	2.97 A	2.82 BC	2.78C	2.70 D	
Total flavonoids	Control	0.235 bcd	0.232 bcd	0.226 bcd	0.218 d	0.202 e	0.223C
(mg CE/g DM)	2.2 kj	0.245 b	0.239 c	0.234 bcd	0.228 bcd	0.226 bcd	0.234 B
	8.8 kj	0.245 b	0.244 b	0.239 bc	0.234 bcd	0.231 bcd	0.239 AB
	16.4 kj	0.266 a	0.245 b	0.241 bc	0.235 bcd	0.234 bcd	0.244 A
	Overall	0.248 A	0.240 AB	0,235BC	0.229 CD	0.223 D	
Total carotenoids	Control	4.72 a	4.47 b	4.34 c	3.74 d	3.69 e	4.19 A
(mg/100 g DM)	2.2 kj	3.68 e	3.64 f	3.62 g	3.33 h	3.30 ı	3.51 B
	8.8 kj	2.74 j	2.67 k	2.65 l	2.35 n	2.32◦	2.54C
	16.4 kj	2.64 l	2.60 m	2.58 m	2.30 p	2.24 q	2.47 D
	Overall	3.44 A	3.34 B	3.30C	2.93 D	2.89 E	
Ascorbic acid	Control	0.33 ı	0.25 j	0.24 j	0.23 j	0.23 j	0.26 D
(g/100 g DM)	2.2 kj	0.55 de	0.44 g	0.42 hg	0.38 hı	0.37 ı	0.43C
	8.8 kj	0.60 c	0.62 bc	0.54 ef	0.52 ef	0.50 f	0.56 B
	16.4 kj	0.71 a	0.67 b	0.66 b	0.65 b	0.59 cd	0.66 A
	Overall	0.55 A	0.50 B	0.46 C	0.45 C	0.42 D	
EC_{50} values[c]	Control	0.32 e	0.45 c	0.47 bc	0.47 bc	0.43 cd	0.43C
(mg DM/mg DPPH)	2.2 kj	0.38 d	0.51 ab	0.50 ab	0.51 ab	0.51 ab	0.48 B
	8.8 kj	0.39 d	0.51 ab	0.51 ab	0.51 ab	0.51 ab	0.49 B
	16.4 kj	0.44 c	0.52 a	0.51 ab	0.52 a	0.54 a	0.51 A
	Overall	0.38 B	0.50 A	0.50 A	0.50 A	0.50 A	

[a] Means in the same row with different letters are significantly different at $P \leq 0.05$ by Duncan's multiple range test.
[b] Means in the same column with different letters are significantly different at $P \leq 0.05$ by Duncan's multiple range test.
[c] EC_{50} of Trolox was determined as 0.12 ± 0.01 mg mg DPPH^{-1}.

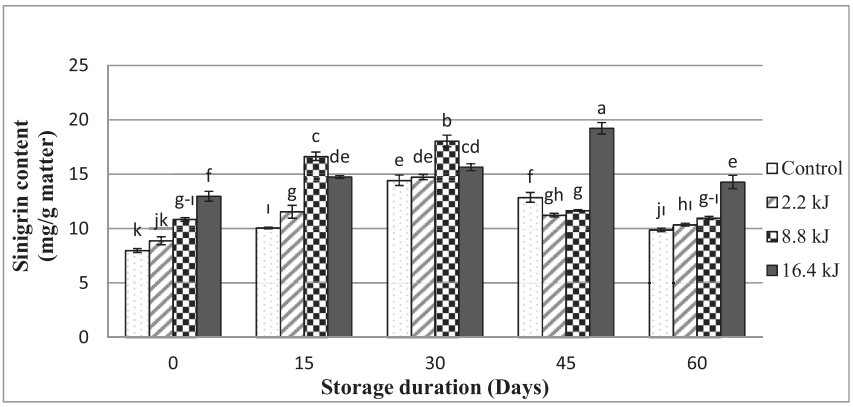

Fig. 3. The effects of different UV radiation doses on sinigrin content of 'Naxos F_1' broccolis during storage at 0 °C. Data represent the mean values from three independent experiments ± standard deviation (SD). Different letters above the columns indicate significant differences at $P < 0.05$.

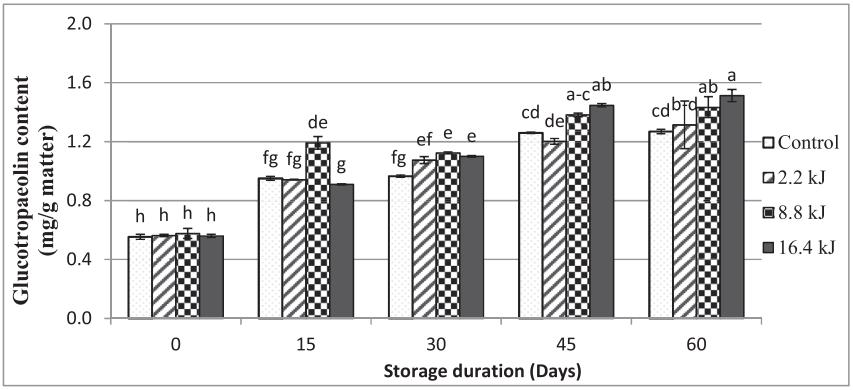

Fig. 4. The effects of different UV radiation doses on glucotropaeolin content of 'Naxos F_1' broccolis during storage at 0 °C. Data represent the mean values from three independent experiments ± standard deviation (SD). Different letters above the columns indicate significant differences at $P < 0.05$.

acid levels 15 days after germination of bean plants. In contrast, a reduction in ascorbic acid was observed 30 days after germination, indicating the temporal character of UV-induced effects on metabolites such as ascorbic acid.

3.9. Sinigrin and glucotropaeolin content

Sinigrin (2-propenyl glucosinolate) (Fig. 3) and glucotropaeolin (benzyl glucosinolate) (Fig. 4) contents of control broccoli samples were determined to be 7.97 mg/g DM and 0.55 mg/g DM, respectively. The initial sinigrin content of the samples was increased when plants had been exposed to UV ($P < 0.05$) while there was no significant change in glucotropaeolin content of the samples at the beginning of storage. Mevis et al. (2012) have reported two-fold increases in aliphatic glucosinolates levels in broccoli sprouts after exposure to UV-B compared with non-irradiated sprouts. They concluded that it is not clear whether the glucosinolates are synthesized de novo in the sprouts or if they are translocated from the roots to the aerial part of the plant. They also stated that UV-B might slow down the rate of glucosinolates degradation in sprouts and that the rate of degradation might be inversely correlated with the UV-B dose.

Sinigrin contents of the samples tended to increase during the first month of storage. The highest sinigrin levels were detected in the samples treated with 16.4 kJ/m^2 UV, and a peak level of 18.60 mg/g DM sinigrin was found after 30 days storage. After that day, sinigrin content of the samples gradually decreased (on average by 28%) till the end of the storage period. The reduction in the sinigrin content after 30 days storage can be related to the hydrolysis of this compound during storage (Mevis et al., 2012). The glucotropaeolin contents of the samples, however, substantially increased during storage, which can be caused by transformation of other similar metabolites to glucotropaeolin. The highest glucotropaeolin level was 1.51 mg/g DM for the samples, and this was found in heads raised under 16.4 kJ/m^2 UV, after 60 days storage.

In conclusion, this study is one of the first reports on antioxidant compounds, antioxidant activity and postharvest quality of broccolis following supplementary UV exposure during the vegetative growth and development phase. In this study, broccoli heads exhibited profound changes in color, appearance and antioxidant activity during their storage life. Moreover, the supplementary UV radiation during plant growth significantly decreased the chlorophyll a, chlorophyll b and carotenoid but increased ascorbic acid, total phenolics and flavonoids. UV treatment slightly reduced antioxidant activity, however, no remarkable change was observed between 2.2 and 8.8 kJ/m^2 radiation levels. Sinigrin and glucotropaeolin contents of the broccolis were substantially increased by UV treatments. The prolonged storage period has resulted in a decrease in ascorbic acid, total phenolic and flavonoid contents, and antioxidant activity as well. Therefore, it is concluded that UV exposure during the vegetative period is a promising tool to increase the beneficial health components of broccoli heads.

Acknowledgment

The authors wish to acknowledge the financial support given by

(TUBITAK) the Scientific and Technological Research Council of Turkey (grant number: TOVAG 112O352). The authors thank to Dr. Wagdy Sobeih and Dr. Marcel A.K. Jansen for critical review of the manuscript.

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