Impact of UV-B irradiation on chlorophyll degradation and chlorophyll-degrading enzyme activities in stored broccoli (*Brassica oleracea* L. Italica Group) florets

Sukanya Aiamla-or[a], Samak Kaewsuksaeng[b**], Masayoshi Shigyo[a,b], Naoki Yamauchi[a,b*]

[a] The United Graduate School of Agricultural Science, Tottori University, Koyama-Minami, Tottori 680-8553, Japan

[b] Faculty of Agriculture, Yamaguchi University, Yoshida, Yamaguchi 753-8515, Japan

* Corresponding author Tel.: +81-83-933-5843; Fax: +81 83933 5820; E-mail address:yamauchi@yamaguchi-u.ac.jp

**Present address: Faculty of Technology and Community Development, Thaksin University, Phatthalung campus, Phatthalung 93110, Thailand

ABSTRACT

UV-B irradiation was applied to broccoli florets to investigate its effect on chlorophyll degradation and chlorophyll-degrading enzyme activities in stored broccoli. Broccoli florets were irradiated with UV-B doses at 4.4, 8.8 and 13.1 kJ m^{-2} and then kept at 15 ºC in darkness. We found that a UV-B dose of at least 8.8 kJ m^{-2} efficiently delayed the decrease of the hue angle value and the contents of chlorophylls *a* and *b*. Chlorophyllide *a* and 13^2-hydroxychlorohyll *a* gradually decreased with senescence. Pheophorbide *a* and pyropheophorbide *a* levels were significantly higher in broccoli without UV-B treatment. Chlorophyllase and chlorophyll-degrading peroxidase activities with UV-B treatment were suppressed, as well as the activity of Mg-dechelatase. Mg-dechelating substance activity was also suppressed with this treatment. We concluded that UV-B treatment effectively suppressed chlorophyll degradation in broccoli florets during storage, suggesting that the effect could be due to the suppression of chlorophyll-degrading enzyme activities.

Keywords: Broccoli; UV-B; Chl degradation; Chl derivatives; Chl-degrading enzymes

1. Introduction

Broccoli is a highly perishable product and floret yellowing is a major limitation to shelf life and quality. Therefore, suitable treatments are necessary to maintain quality levels until consumption. Some techniques used to delay senescence include heat treatments, which effectively reduce yellowing among stored broccoli florets (Funamoto, Yamauchi, Shigenaga & Shigyo, 2002; Costa, Civello, Chaves & Martínez, 2006; Kaewsuksaeng, Yamauchi, Funamoto, Mori, Shigyo & Kanlayanarat, 2007); chemical treatments, such as 1-methylcyclopropene (Able, Wong, Prasad & O'Hare., 2002) and ethanol vapor (Suzuki, Uji & Terai, 2004); plant hormone treatment like a cytokinin (Costa, Civello, Chaves & Martínez, 2005); low temperature storage (Starzyńska, Leja & Mareczek, 2003); and controlled atmosphere storage (Yamauchi & Watada, 1998). Recently, treatment with UV-C was reported to maintain the postharvest quality of strawberries (Erkan, Wang & Wang, 2008) and also to inhibit chlorophyll (Chl) degradation in stored broccoli florets (Costa, Vicente, Civello, Chaves & Martínez, 2006). However, the effects of UV-A and/or UV-B on Chl degradation in stored broccoli florets have not been clarified. Previous studies reported that UV-A and UV-B irradiation enhanced the level of antioxidant compounds and antioxidant enzyme activity in plants (Costa, Gallego & Tomaro, 2002). Toivonen & Sweeney (1998) reported that antioxidant enzymes containing superoxide dismutase, peroxidase and catalase are important for the retardation of Chl degradation in broccoli. However, no study has examined the effect of the postharvest application of UV-A and/or UV-B on Chl degradation in broccoli florets. Furthermore, UV-A and UV-B are less harmful wavelengths, in comparison with UV-C,

and may represent a new practical approach for maintaining the postharvest quality of fruits and vegetables.

The early steps of Chl degradation include the removal of phytol and the formation of chlorophyllide (Chlide) *a* by chlorophyllase (Chlase) (Harpaz-Saad et al, 2007), followed by the removal of an Mg atom by either Mg-dechelatase (MD) (Langmeier, Ginsburg & Matile, 1993) or another Mg-dechelating substance (MDS) (Shioi, Tomita, Tsuchiya & Takamiya, 1996). Finally, pheophorbide (Pheide) *a* is degraded to fluorescent Chl catabolites, which are primarily colorless catabolites, via red Chl catabolite by Pheide *a* oxygenase and red Chl catabolite reductase (Matile, Hörtensteiner & Thomas, 1999). Chl *a* is also degraded *in vitro* by Chl-peroxidase (POX) in the presence of some phenolic compounds and hydrogen peroxide to form 13^2-hydroxychlorohyll (OHChl) *a*, which is an oxidized form of Chl *a* (Yamauchi, Funamoto & Shigyo, 2004). In horticultural crops, OHChl *a* is usually presents as a Chl derivative, and its content shows a decline with senescence during storage (Yamauchi & Watada, 1991, 1993, 1998).

Previously, we have investigated the effects of UV-A and UV-B treatment on yellowing of broccoli florets during storage at 15 ºC. In general, broccoli florets retained more color after UV-B irradiation as compared to UV-A irradiation, although the doses of UV-A treatment and UV-B treatment were similar (Aiamla-or, Yamauchi & Shigyo, 2007) Here, we therefore examined the impact of UV-B irradiation on Chl degradation and Chl-degrading enzyme activities and the resultant quality control of broccoli using UV-B irradiation.

2. Materials and Methods

2.1. Plant materials and UV-B treatments

Broccoli (*Brassica oleracea* L. cv. endeavor) heads were harvested in the Fukuoka Prefecture and transported to the Horticultural Science laboratory at Yamaguchi University. Broccoli heads were immediately irradiated with UV-B (spectral peak value: 312 nm, T-15M, VL). Each broccoli head was placed vertically under the UV-B lamps at a distance of 15 cm, resulting in UV-B energy doses of 4.4, 8.8 and 13.1 kJ m^{-2}. Broccoli florets were kept in polyethylene film bags (0.03 mm in thickness), with the top folded over. The bags were then placed on a plastic tray and stored at 15 °C in the dark. Triplicates of three heads were removed at scheduled intervals during the 6-day storage period, and the floral tissue was analyzed.

2.2. Surface color and Chlorophyll assays

Chl content was determined using *N*,*N*-dimethylformamide (Moran, 1982). The surface color of the heads, as represented by hue angle, was measured with a color difference meter (Nippon-denshoku NF 777).

2.3. Preparation of substrates

2.3.1. Chlorophyll a

Spinach leaves were homogenized for 3 min in cold acetone (–20 °C). The homogenate was filtrated through two layers of Miracloth (Calbiochem, USA). The filtrates were treated with dioxane and distilled water and then kept for 1 h on ice. The filtrates were centrifuged at 10,000 × *g* for 15 min at 4 ºC. After centrifugation, the pellets were treated again with acetone, dioxane and distilled water, and then kept for 1 h on ice. Afterwards, the soluble pellets were centrifuged at 10,000 × *g* for 15 min at 4 ºC and where subsequently dissolved in petroleum ether. Soluble chlorophyll in petroleum ether was stored at –20 °C until the individual pigments were separated using sugar powder column chromatography (Perkins & Roberts, 1962). Finally, five hundred µg/mL of Chl *a* was prepared in acetone.

2.3.2. Chorophyllin a

Chlorophyllin (Chlin) *a* was slightly modified according to Vicentini, Iten & Matile (1995). The Chl *a* acetone solution (500 µg/mL) was partitioned into petroleum ether. The petroleum ether phase was washed three times with 20 mL of distilled water, after which 30% KOH in methanol was mixed into the solution. The Chlin *a* was allowed to precipitate and was then centrifuged at 16,000 × *g* at 4 ºC for 15 min. The precipitate was dissolved in distilled water and adjusted to pH 9.0 with 2 M of tricine.

2.3.3. Chlorophyllide a

Chlorophyllide (Chlide) *a* was prepared from a Chl *a* acetone solution (500 μg/mL) with 0.798 mg protein of partial purified Chlase (20-40% of $(NH_4)_2SO_4$) from green citrus fruits. The reaction mixture was incubated at 25 °C for 40 min. The reaction was stopped using acetone and the remaining Chl *a* was separated by hexane. The lower part of the reaction mixture was used as the Chlide *a*.

2.4. Analyses of Chlorophyll-degrading enzyme activities

An acetone powder (500 mg) of floral tissues was suspended in 15 mL 10 mM phosphate buffer (pH 7.0) containing 0.6% CHAPS for Chlase. For Mg-dechelatase, an acetone powder (500 mg) of floral tissues was suspended in 15 mL 50 mM phosphate buffer (pH 7.0) containing 50 mM KCl and 0.24% Triton-X 100, or in 15 mL 10 mM phosphate buffer (pH 7.0) for Chl-POX. The crude enzyme was stirred for 1 h at 0 °C and the mixture was filtered with two layers of Miracloth. The filtrate was then centrifuged at 16,000× *g* at 4 °C for 15 min. The supernatant was used as the crude enzyme extract. The enzyme protein contents were determined based on Bradford's method (1976).

2.4.1. Chlorophyllase activity

The reaction mixture contained 0.5 mL 0.1 mM phosphate buffer (pH 7.5), 0.2 mL 500 μg/mL Chl *a* acetone solution (100 μg/mL) and 0.5 mL enzyme solution. The reaction mixture was incubated in a water bath at 25 °C for 40 min, and the enzyme reaction was stopped by adding 4 mL of acetone. Chlide *a* was separated by adding 4 mL

of hexane. The upper phase contained the remaining Chl *a* while the lower phase contained the Chlide *a*. The activity was spectrophotometrically detected by Chlide *a* formation at 667 nm per unit per mg protein.

2.4.2. Chlorophyll-degrading peroxidase activity

Chl-POX was determined as previously described (Yamauchi, Harada & Watada., 1997). The reaction mixture contained 0.5 mL of enzyme solution, 0.1 mL 1.0% Triton X-100, 0.1 mL 5 mM *p*-coumaric acid, 0.2 mL 500 µg/mL Chl *a* acetone solution, 1.5 mL 0.1 mM phosphate buffer (pH 5.5) and 0.1 mL 0.3% hydrogen peroxide. Activity was determined spectrophotometrically by measuring the decrease of Chl *a* at 668 nm per unit per mg protein at 25 °C.

2.4.3. Mg-dechelatase activity

Mg-dechelatase activity using Chlin *a* was determined spectrophotometrically by measuring the absorbance of pheophorbin *a* formation at 686 nm (Costa, Gallego & Tomaro, 2002). The reaction mixture, which contained 0.75 mL 50 mM Tris-HCl buffer (pH 8.0), 0.3 mL Chlin *a* and 0.2 mL of enzyme solution, was incubated at 37 ºC. Mg-dechelatase activity using Chlide *a* was determined by the method of Suzuki & Shioi (2002) with slight modification. The activity of Mg-dechelatase was measured with Pheide *a* formation, the reaction mixture contained 0.75 mL 10 mM phosphate buffer (pH 7.5), 0.25 mL Chlide *a* (7.55 µg) and 0.2 mL of enzyme solution.

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183 *2.5. Analyses of Chlorophyll and resulting derivatives*

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185 The acetone-HEPES solution was prepared from 16 mL of cold acetone and 2 mL
186 50 mM HEPES buffer (pH 7.5). Two grams of fresh broccoli florets were ground in 9 mL
187 of acetone-HEPES solution using a mortar and pestle and the remaining solution (9 mL
188 acetone-HEPES solution) was then added. The extraction was kept in the dark for 5 min,
189 and passed through filter paper (Whatman # 2). Subsequently, the aliquots were filtered
190 through a DISMIC filter (0.45 µm, AVANTEC, Japan) and then used for HPLC analyses.
191 Chl and the resulting derivatives were analyzed by HPLC using a Hitachi L-700 pump
192 with an automated gradient controller and a Hitachi L-2450 diode array detector or a
193 Hitachi L-7240 UV-visible spectrophotometer. The absorption spectrum of the pigment
194 was recorded at 665 nm. Pigments were separated on a LiChropher C18 column
195 (MERCK), 4×250 mm, using two solvents; solvent A, 80% methanol (methanol:milipore
196 water, 80:20, v/v) and B, 100% ethyl acetate in a gradient. Solvent B was added to
197 solvent A at a linear rate until a 50:50 mixture was attained at the end of 20 min. The
198 50:50 mixture was then used isocratically for an additional 20 min, as described by Eskin
199 & Harris (1981). The flow rate was 1.0 mL/min, and the injection volume was 100 µL.
200 The identification of Chl and the resulting derivatives were based on the retention time
201 and the visible absorption spectra. Chl derivative standards, such as Pheide *a* and
202 Pyropheide *a* were purchased from Wako Pure Chemical Industries (Tokyo, Japan), and
203 Tama Biochemical (Tokyo, Japan), respectively. Phy *a* standard was prepared by adding
204 a few drops of 0.1 M hydrochloric acid to Chl *a* solution (Holm-Hansen, Lorenzen,

Holmes & Strickland, 1965). OHChl *a* was prepared by adding peroxidase (horseradish, Sigma-Aldrich, USA) into Chl *a* solution in the present of hydrogen peroxide and *p*-coumaric acid, as described by Kaewsuksaeng, Yamauchi, Funamoto, Mori, Shigyo & Kanlayanarat (2007).

3. Results

3.1. Optimization of UV-B dose for broccoli floret treatments

As shown in Fig. 1, hue angle value was not altered in broccoli florets during the first two days of storage. However, a decrease in the hue angle value was found on day 4. Broccoli in the control showed lowest hue angle value. We found that the decrease of the hue angle value was delayed by a UV-B dose at least 8.8 kJ m^{-2}. The hue angle values were high in broccoli with 8.8 and 13.1 kJ m^{-2} of UV-B on day 6, as compared to florets in the control and those irradiated with 4.4 kJ m^{-2}. UV-B treatment delayed the reduction of total Chl content, as shown in Fig. 2A. Moreover, a UV-B dose of at least 8.8 kJ m^{-2} delayed the degradation of Chl *a* in broccoli florets. As apparent in Fig. 2B, Chl *a* content in fresh broccoli was approximately 94 mg/100g FW, but during storage, the content greatly decreased without UV-B treatment and with UV-B doses at 4.4 kJ m^{-2} by 19 and 13 mg/100g FW, respectively. On the other hand, Chl *a* content in broccoli treated with UV-B doses at 8.8 and 13.1 kJ m^{-2} retained approximately 33 and 25 mg/100g FW, respectively, on day 6 of the storage. In the case of Chl *b*, the alteration trend of its content was similar to that of Chl *a* content in broccoli during storage. In Fig. 2C, the

228 broccoli treated with a UV-B dose at least 8.8 kJ m^{-2} had a higher content of Chl *b* than
229 the broccoli without UV-B treatment on day 4. Based on the results of the hue angle
230 values and Chl contents, UV-B treatment at a dose of 8.8 kJ m^{-2} was selected to further
231 analyze the impact of UV-B treatment on the formation of Chl derivatives and Ch-
232 degrading enzyme activities in stored broccoli florets.
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234 *3.2. Changes in Chlorophyll derivatives*
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236 As shown in Fig. 3. fresh broccoli florets had high Chlide *a* levels compared to
237 stored broccoli. The Chlide *a* levels gradually decreased in broccoli without UV-B
238 treatment, but the decrease in Chlide *a* levels was delayed in broccoli with UV-B
239 treatment (Fig. 3A). In Fig. 4, showing the Chl derivative chromatogram, broccoli
240 without UV-B treatment can be seen to have a lower Chlide *a* level than broccoli with
241 UV-B treatment on day 4. Broccoli with UV-B treatment also showed a high level of
242 OHChl *a* as compared to broccoli without UV-B treatment. The trend in OHChl *a* levels
243 was similar to the Chlide *a* level in broccoli, as shown in Fig. 3B. Pheide and Pyropheide
244 *a* were found in stored broccoli on day 4. In broccoli without UV-B treatment, both
245 Pheide and Pyropheide *a* levels sharply increased during storage, whereas broccoli with
246 UV-B treatment showed a slight increase. Although, the levels of Pheide and Pyropheide
247 *a* greatly increased in broccoli without UV-B treatment on day 4, the levels of both
248 Pheide *a* and Pyropheide *a* decreased in stored broccoli on day 6. In contrast, broccoli
249 treated with UV-B showed a continuous increase in Pheide *a* and Pyropheide *a* levels
250 (Fig. 3C, D). The Phy *a* level in fresh broccoli florets was higher than any other

derivative level. Broccoli with UV-B treatment showed a higher level of Phy *a* than that without UV-B treatment throughout the storage period. Moreover, Phy *a* level was found to be slightly increased in broccoli with UV-B treatment at day 4, followed by a decrease in the level on day 6 (Fig. 3E).

3.3. Changes in Chlorophyll-degrading enzyme activities

In Fig. 5A, Chlase activity in broccoli with or without UV-B treatment decreased during storage. Fresh broccoli florets had high Chlase activity compared to stored broccoli. Chlase activity in broccoli without UV-B treatment was approximately 0.17 units/mg protein, but its activity was lowered in broccoli with UV-B treatment. Notably, the enhancement of Chlase activity was suppressed by UV-B treatment during the first two days of storage. During the first two days, Chl-POX activity slightly increased in both the control and the UV-B treatments, and then sharply increased only in broccoli without UV-B treatment on day 6. In contrast, Chl-POX activity showed almost no change in broccoli with UV-B treatment after 2 days of storage (Fig 5B). In this study, Mg-dechelation activity was examined by using Chlin *a* as an artificial substrate, tentatively named Mg-dechelatase (MD). After UV-B treatment, MD activity was not at a significantly different level between the control and the UV-B treatment during the first 4 days of storage. However, MD activity was suppressed by UV-B treatment, as its activity greatly increased in broccoli without UV-B treatment on day 6 (Fig. 5C). In contrast, Mg-dechelation activity using Chlide *a* as a native substrate, tentatively named Mg-dechelating substance (MDS), showed unchanged in broccoli on day 6 of the storage. In

addition, the UV-B treatment effectively reduced the activity of MDS in broccoli during storage (Fig. 6).

4. Discussion

Broccoli is a cole crop with floral heads consisting of small florets arranged on branches sprouting from stalk. When broccoli heads are harvested, their florets are immature and in the phase of their most intense growth, which makes them very sensitive to stress factors and leads to a rapid initiation of senescence. Senescence in broccoli is normally characterized by a decrease in pigment, as well as Chl degradation (Yamauchi & Watada, 1998). Several techniques have been applied to maintain the green color of broccoli florets (Funamoto, Yamauchi, Shigenaga & Shigyo, 2002; Costa, Civello, Chaves & Martínez, 2005, 2006; Costa, Vicente, Civello, Chaves & Martínez, 2006). In the present study, different UV-B doses (4.4, 8.8 and 13.1 kJ m^{-2}) were irradiated into broccoli florets. We found that a UV-B dose of at least 8.8 kJ m^{-2} effectively delayed the yellowing of florets and Chl degradation. Based on the results of hue angle value and Chl degradation, we suggested that 8.8 kJ m^{-2} was an optimal UV-B dose and used this does for further study. The delay of Chl degradation with UV-B treatment may have the same effect as heat treatment (Funamoto, Yamauchi, Shigenaga & Shigyo, 2002; Costa, Civello, Chaves & Martínez, 2006) and UV-C irradiation (Costa, Vicente, Civello, Chaves & Martínez, 2006), which also suppressed Chl-degrading enzyme activities in broccoli florets. In this study, a UV-B dose at 8.8 kJ m^{-2} effectively suppressed the activities of Chl-degrading enzymes such as Chlase, Chl-POX, MD and MDS, in broccoli

297 florets. During storage, Chlase activity decreased in broccoli florets with or without UV-
298 B treatment, and it was previously reported that Chlase activity decreased with the
299 senescence of leaves (Ben-Yaakov, Harpaz-saad, Galili, Eyal & Goldschmidt, 2006). In
300 the study, we found that Chlase activity was tentatively suppressed in stored broccoli
301 florets during the first two days of storage by UV-B treatment. Chlase, which is involved
302 in the first step of Chl catabolic pathway, catalyzes the conversion of Chl *a* to Chlide *a*
303 and phytol (Harpaz-Saad et al, 2007). The highest level of Chlide *a* was found in fresh
304 broccoli florets and it decreased in stored broccoli. As might be expected a decrease in
305 Chlide *a* is associated with the noted decrease in Chlase activity during storage. After 4
306 days, the level of Chlide *a* was highly retained in broccoli with UV-B treatment as
307 compared to broccoli without UV-B treatment. This might be due to UV-B treatment
308 effectively suppressing Chlase activity, and also delaying the reduction of Chlide *a* levels
309 in broccoli. Our results showed that Chl-POX activity was markedly increased in broccoli
310 during storage, but its activity was clearly suppressed throughout the storage life of
311 broccoli treated with UV-B. In broccoli, Chl *a* can be degraded by Chl-POX, Chl oxidase
312 and lipoxygenase, resulting in OHChl *a* (Lüthy, Martinoia, Matile & Thomas, 1984;
313 Yamauchi, Funamoto & Shigyo 2004). OHChl *a* is formed as an intermediate and does
314 not accumulate. Therefore, in horticultural crops, the content of OHChl *a* usually show a
315 decrease with senescence during storage (Yamauchi & Watada, 1991, 1993, 1998).
316 Notably, the decrease of OHChl level was delayed by UV-B treatment after 4 days of
317 storage. In addition, Pheide *a* and Pyropheide *a* levels in broccoli with UV-B treatment
318 were slowly accumulated as compared to broccoli without UV-B treatment.

It is possible that UV-B treatment could not only effectively delay Chl degradation in broccoli but also retard the other senescence processes that occur during storage. It is known that both antioxidative components and antioxidative enzyme activities increase by UV irradiation (Costa, Gallego & Tomaro, 2002). Toivonen & Sweeney (1998) reported that superoxide dismutase, peroxidase and catalase are important for the retardation of Chl degradation and senescence in broccoli. Furthermore, UV-B treatment also effectively suppressed MDS and MD activities in broccoli florets. However, UV-B doses at 4.4 kJ m^{-2} resulted in the florets quickly turning yellow as compared to other UV-B doses. In this case, the acceleration of broccoli senescence may be cased by a certain level of UV-B dosage. From the results of our study, the optimal dose of UV-B treatment delayed Chl degradation in broccoli florets by the suppression of the Chl-degrading enzyme activities. However, the impact of UV-B irradiation in delaying the Chl degradation of broccoli and its relationship with the antioxidant system need to be further investigated. In addition, Mg-dechelation activity was determined by using Chlin *a* and Chlide *a* as artificial and native substrates. As above mentioned, we tentatively called these Mg-dechelatase (MD) and Mg-dechelating substance (MDS), respectively. Especially, the MD activity increased in broccoli during storage. However, MDS activity was consistently unchanged in stored broccoli florets. These findings were similar to the finding by Suzuki, Kunieda, Murai, Morioka & Shioi (2005) that MDS activity was not altered in radish cotyledons. Furthermore, MD acted only on the frequently used artificial substrate, Chlin *a*, but MDS, which is small molecule and heat stable substance, was required to remove the magnesium atom from Chlide *a*. Accordingly, we suggest that

MDS could be involved in Mg-dechelation from Chlide *a* in broccoli florets. Further study needs to clarify the role of MDS in Chl degradation of broccoli florets.

In conclusion, the findings obtained in the present study show that UV-B dosage of at least 8.8 kJ m^{-2} effectively retarded the degradation of Chl in broccoli florets during storage. The reduction of Chl derivative levels, such as Chlide and OHChl *a*, were retarded by a 8.8 kJ m^{-2} of UV-B dose. Furthermore, UV-B treatment effectively delayed the accumulations of Pheide and Pyropheide *a* in stored broccoli florets. Chl-degrading enzyme activities such as Chlase, Chl-POX and Mg-dechelation were also suppressed by UV-B treatment, indicating that the suppression of those enzyme activities by UV-B treatment cloud be involved in retardation of Ch degradation in stored broccoli florets. We suggest that UV-B treatment could be a good practical approach for maintaining the postharvest quality of broccoli.

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Figure captions;

Figure 1. Changes in the hue angle value of the surface color of broccoli florets with and without UV-B irradiation during storage at 15 ºC. Broccoli florets were irradiated with UV-B of 4.4, 8.8 and 13.1 kJ m^{-2}. Vertical bars represent the average values with ± SE (n = 3).

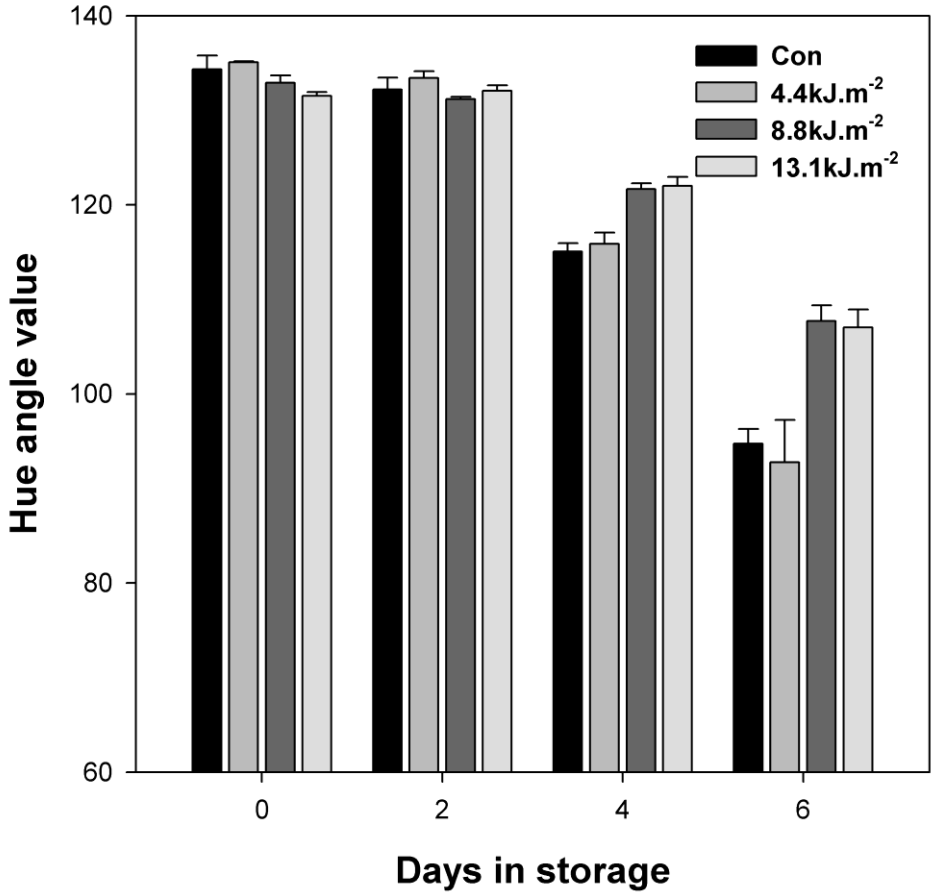
Figure 2. Changes in total Chl (A), Chl *a* (B) and Chl *b* (C) contents in broccoli florets with different doses of UV-B treatment. Broccoli florets were irradiated with UV-B at 4.4, 8.8 and 13.1 kJ m^{-2} and stored at 15 ºC in darkness.

Figure 3. Changes in the Chl derivatives levels (A; Chlide *a*: chlorophyllide *a*, B; OHChl *a*: 13^2-hydroxychlorophyll *a*, C; Pheide *a*: pheophobide *a*, D; Pyropheide *a*: pyropheophobide *a* and E; Phy:pheophytin *a*) of broccoli florets with and without UV-B doses of 8.8 kJ m^{-2} during storage at 15 °C. Chl derivatives were analyzed using HPLC system. Vertical bars represent average values with ± SE (n = 3).

Figure 4. HPLC chromatograms of Chl and Chl derivatives (Chlide *a*: chlorophyllide *a*, OHChl *a*: 13^2-hydroxychlorophyll *a*, Pheide *a*: pheophorbide *a*, Pyropheide *a*: pyropheophorbide *a* and Phy *a*:pheophytin *a*) in broccoli florets on day 4. Broccoli florets were irradiated with a UV-B dose of 8.8 kJ m^{-2} and then kept into incubator at 15 °C.

Figure 5. Changes of Chl-degrading enzyme activities in broccoli florets with and without UV-B at 8.8 kJ m^{-2} during storage at 15 °C. Vertical bars represent average values with ± SE (n = 3). A; Chlase: chlorophyllase, B; Chl-POX: Chlorophyll-peroxidase and C; MD: Mg-dechelatase using Chlin *a* as an artificial substrate.

Figure 6. Changes in Mg-dechelating substrance (MDS) activity in broccoli florets with and without UV-B at 8.8 kJ m^{-2} during storage at 15 °C, using Chlide *a* as a native substrate. Vertical bars represent average values with ± SE (n = 3)



485

486

487 Figure 1.

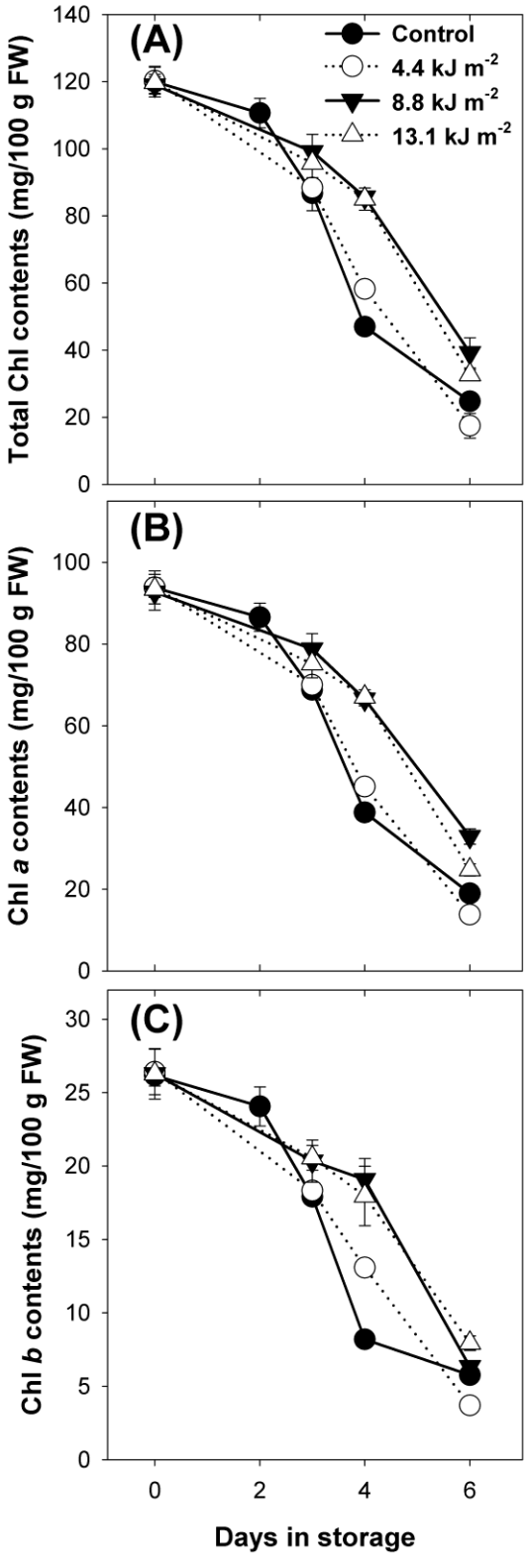
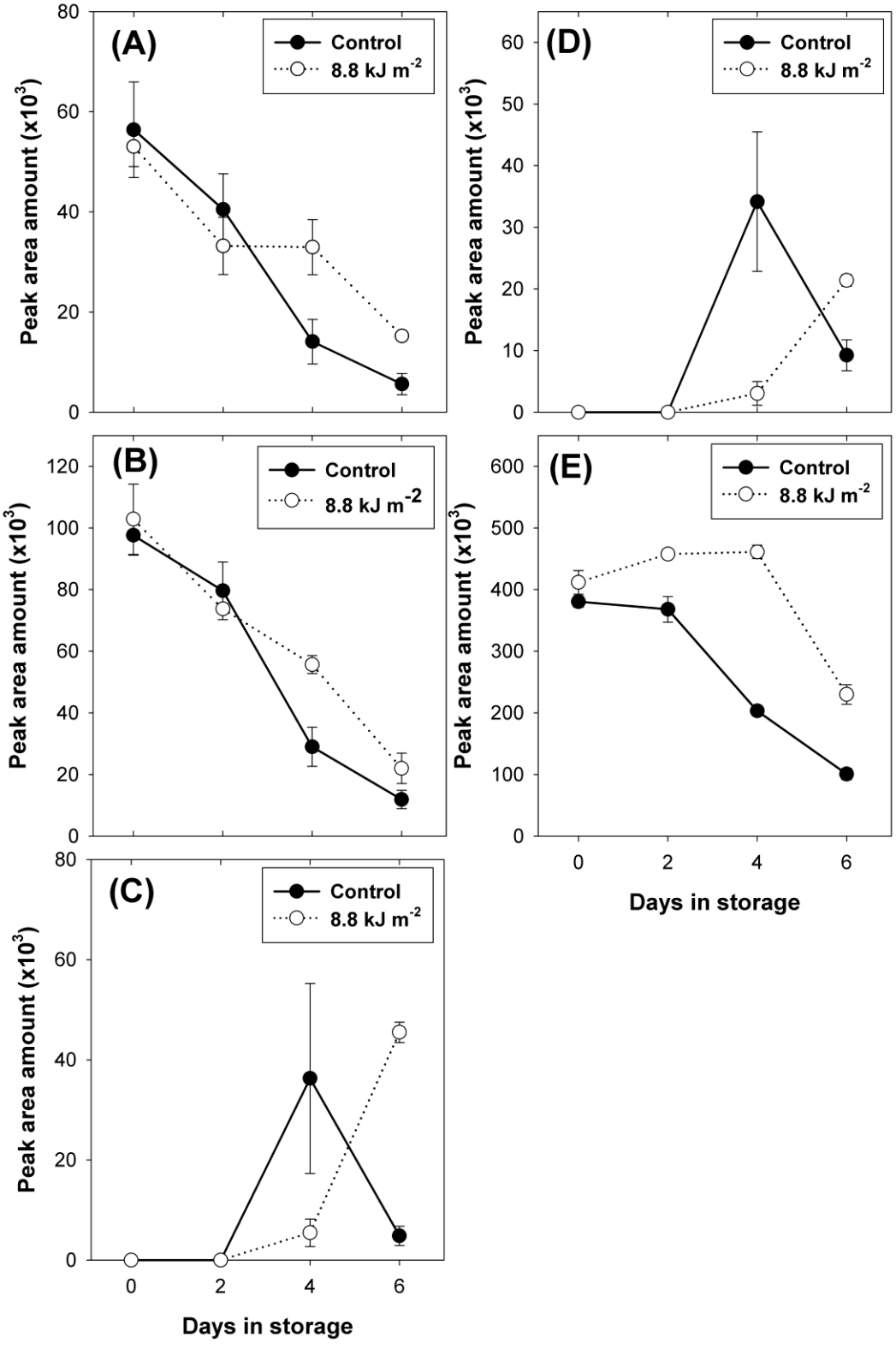

(A)
Control
4.4 kJ m-2
8.8 kJ m-2
13.1 kJ m-2
Total Chl contents (mg/100 g FW)
(B)
Chl a contents (mg/100 g FW)
(C)
Chl b contents (mg/100 g FW)
Days in storage

Figure 2.



490

491 Figure 3

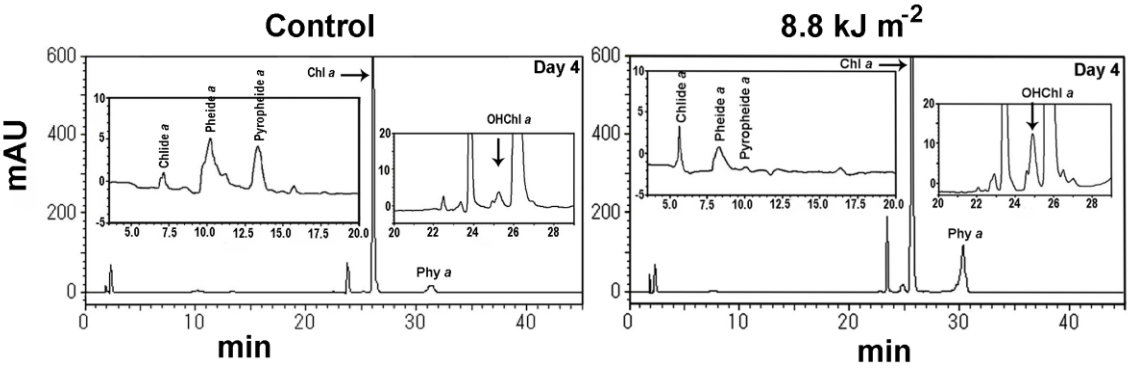
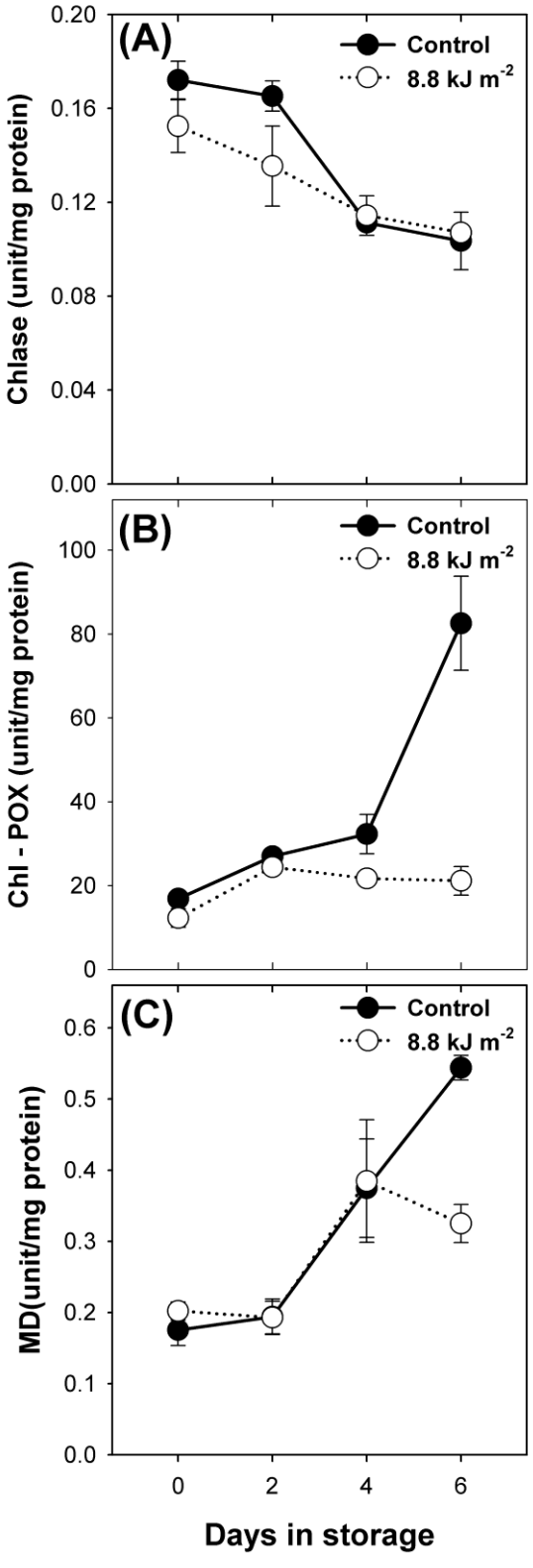

Control
8.8 kJ m-2
mAU
min
Day 4
Chl a
Chlide a
Pheide a
Pyropheide a
OHChl a
Phy a

Figure 4.



496

497 Figure 5.

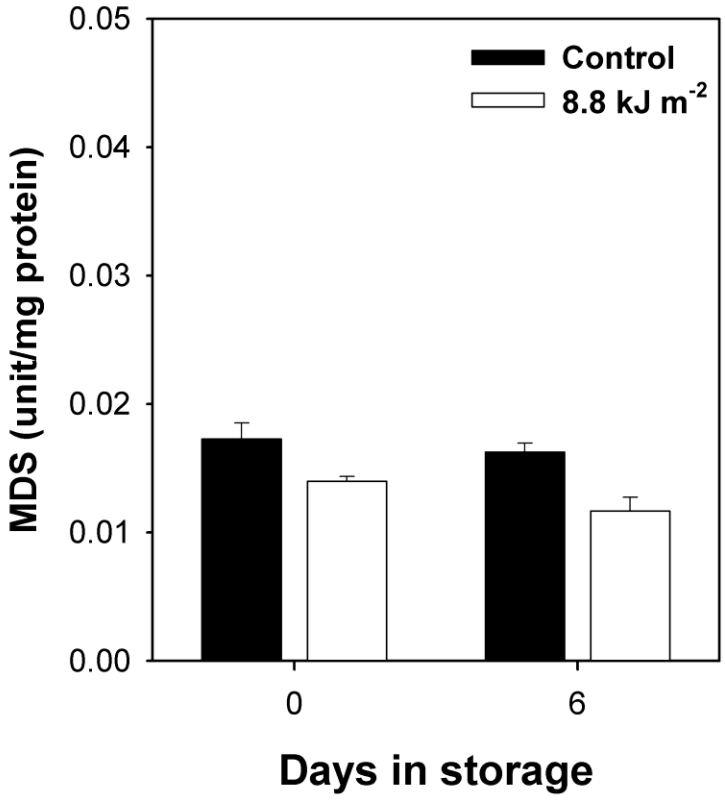

0.05
0.04
0.03
0.02
0.01
0.00
MDS (unit/mg protein)
Control
8.8 kJ m-2
0
6
Days in storage

Figure 6.