1	Effect of UV-A and UV-B irradiation on broccoli (Brassica oleracea L. Italica Group)
2	floret yellowing during storage
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1 Abstract

3	UV-A or UV-B irradiation was applied to broccoli florets to investigate their effect on
4	floret yellowing. Broccoli florets were irradiated with two UV-A doses (4.5 and 9.0 kJ m ⁻
5	²) and five UV-B doses (4.4, 8.8, 13.1, 17.5, and 26.3 kJ m ^{-2}) and then kept in darkness at
6	15 °C. In general, broccoli florets retained more color after UV-B irradiation as compared
7	to UV-A irradiation. UV-B doses of at least 8.8 kJ m^{-2} to broccoli florets resulted in
8	surface color with a higher hue angle, as compared to those treated with 4.4 kJ m^{-2} UV-B
9	or without UV-B. We therefore selected a UV-B dose of 8.8 kJ m^{-2} for application to
10	different broccoli cultivars ('Pixel' and 'Sawayutaka'), harvested during the winter and
11	early summer seasons. During storage, the 'Sawayutaka' cultivar exhibited a slower
12	decrease in green color of florets, when compared to the 'Pixel' cultivar. UV-B treatment
13	delayed floret yellowing and chlorophyll degradation. Broccoli harvested in winter or
14	early summer and irradiated with UV-B during storage at 15 °C displayed higher
15	chlorophyll content and hue angle value than broccoli without UV-B treatment. These
16	results suggest that UV-B irradiation is effective in retaining the green color of florets
17	during storage.
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19	Keywords: UV-A, UV-B, Chlorophyll degradation, Broccoli florets

1. Introduction

1	Floret yellowing is a major limitation to shelf life and broccoli quality. Therefore,
2	suitable treatments are necessary to maintain quality levels until consumption. Some
3	techniques to delay senescence have been investigated, including heat treatments, which
4	effectively reduce yellowing among stored broccoli florets (Funamoto et al., 2002; Costa,
5	et al., 2006; Kaewsuksaeng et al., 2007); chemical treatments such as 1-
6	methylcyclopropene (Ku and Will, 1999; Able et al., 2002) and ethanol vapor (Suzuki et
7	al., 2004); low temperature (Starzyńska et al., 2003); and controlled atmosphere storage
8	(Yamauchi and Watada, 1998). Recently, UV-C irradiation was applied to broccoli
9	florets and effectively delayed floret yellowing during storage (Costa et al., 2006;
10	Lemoine et al., 2008). However, the effects of UV-A and UV-B on yellowing in stored
11	broccoli have not been clarified. Previous studies reported that UV-A and UV-B radiation
12	enhanced the level of antioxidant compounds and antioxidant enzyme activity in plants
13	(Costa et al., 2002; Gao and Zhang, 2008; Xu et al., 2008). However, no study has looked
14	at the effect of postharvest application of UV-A and/or UV-B on the yellowing of
15	broccoli florets. Furthermore, UV-A and UV-B are less harmful wavelengths, in
16	comparison with UV-C. Therefore, these treatments may represent a new practical
17	approach for maintaining the postharvest quality of fruits and vegetables. Notably, the
18	postharvest life of fruits or vegetables on market shelves can be affected by genotypic
19	variation and environmental conditions during crop development (Toivonen and Sweeney,
20	1998; Tan et al., 1999). Here we examine the impact of UV-A or UV-B irradiation on
21	broccoli floret yellowing. We also discuss the influences of cultivar and harvest season
22	on the UV-B-mediated inhibition of yellowing.

2. Materials and Methods

3	Broccoli (Brassica oleracea L. Italica Group) cultivars, 'Sawayutaka' and 'Pixel',
4	were harvested during winter in Fukouka Prefecture and transported to the laboratory of
5	Horticultural Science at Yamaguchi University. The Pixel cultivar was also harvested
6	during early summer. Broccoli heads were immediately irradiated with UV-A (spectral
7	peak value: 342 nm, F15BLB) or UV-B (spectral peak value: 312 nm, T-15M, VL). Each
8	broccoli head was placed vertically under the UV-A or UV-B lamps at a distance of 15
9	cm, resulting in UV-A energy of 4.5 and 9.0 kJ m^{-2} and UV-B energy of 4.4, 8.8, 13.1,
10	17.5 and 26.3 kJ m^{-2} . Broccoli florets were kept in polyethylene film bags (0.03 mm in
11	thickness), with the top folded over. The bags were then placed on a plastic tray and
12	stored at 15 °C in the dark. Triplicates of three heads were removed at scheduled intervals
13	during the 6-day storage period, and the floral tissue was analyzed. Chlorophyll (Chl)
14	content was determined using N,N-dimethylformamide (Moran, 1982). Surface color of
15	the heads, as represented by hue angle, was measured with a color difference meter
16	(Nippon-denshoku NF 777).
17	The experiments were conducted in a completely randomized design. The analysis of
18	variance (ANOVA) of data was performed using SAS (Microsoft Corporation). The
19	deference between means of data were compared by lest significant difference at $P < 0.05$.
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21	3. Results and Discussion
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23 3.1. Optimization of UV irradiation

1	As shown in Tables 1 and 2, UV-A treatment did not delay floret yellowing or reduce
2	the hue angle value, although the doses of UV-A (4.5 and 9.0 kJ m ^{-2}) and UV-B (4.4 and
3	8.8 kJ m ⁻²) were similar. Broccoli exposed to 8.8 kJ m ⁻² UV-B displayed more green
4	florets than broccoli exposed to 4.4 kJ m ^{-2} UV-B or without UV-B treatment (the control).
5	UV-B doses of at least 8.8 kJ m^{-2} significantly delayed the reduction of hue angle values
6	for broccoli stored at 15 °C. Therefore, 8.8 kJ m^{-2} was selected as the optimal UV-B dose
7	and applied in the next experiment. We suggest that UV-B treatment is more effective than
8	UV-A irradiation in delaying floret yellowing and that this discrepancy is due to the
9	difference in wavelength. When we exposed florets to 4.4 kJ m^{-2} of UV-B, the florets
10	turned yellow more quickly than when exposed to the other doses of UV-B. Therefore, the
11	acceleration of broccoli senescence may be affected by UV-B dose. UV-B irradiation is
12	known to induce the formation of reactive oxygen species (ROS), such as hydrogen
13	peroxide, superoxide, hydroxyl radical and single oxygen. ROS can cause oxidative
14	damage to membrane lipids, protein and DNA (Foyer et al., 1994). Fortunately, plants
15	protect themselves against UV-B irradiation by accumulating flavonoid compounds, as
16	well as increasing antioxidant production and antioxidative enzyme activity levels
17	(Robberecht and Caldwell, 1983; Jordan, 1996). Therefore, broccoli senescence can be
18	delayed when increases in the levels of reactive oxygen species trigger these defensive
19	mechanisms in florets exposed to optimal doses of UV-B irradiation.

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21 3.2. Influences of cultivar and harvest season on UV-B-mediated inhibition of yellowing

22 Two broccoli cultivars, 'Pixel' and 'Sawayutaka' were harvested during the winter.

23 During storage, 'Pixel' florets displayed yellowing more rapidly than 'Sawayutaka' cultivar

1	florets. UV-B treatment delayed floret yellowing in both 'Pixel' and 'Sawayutaka' cultivars
2	As shown in Table 3, control florets displayed lower surface color of hue angle values, in
3	comparison to florets exposed to UV-B treatment. Chl contents in 'Sawayutaka' florets
4	were slightly higher than those in the 'Pixel' cultivar, although Chl contents in fresh
5	broccoli were not significant difference between 'Pixel' and 'Sawayutaka' cultivars. The
6	decrease in Chl contents was much greater in 'Pixel' than 'Sawayutaka' during storage.
7	Moreover, Chl contents were significant higher in 'Sawayutaka' with UV-B treatment as
8	compared to 'Pixel' with UV-B treatment on day 6. These results indicated that
9	'Sawayutaka' could be responded more dramatically to UV-B treatment than 'Pixel'.
10	We also determined the effect of harvest season on the inhibitory effect of UV-B
11	treatment. As is apparent in Table 4, broccoli harvested in the early summer exhibited rapid
12	floret yellowing, as well as a gradual reduction in Chl content, indicating that surrounding
13	circumstances during growth and development in broccoli might affect the progress of
14	floret senescence after harvest. Broccoli exposed to UV-B exhibited slight decreases in
15	both hue angle value and Chl content. Notably, UV-B treatment effectively inhibited
16	yellowing in broccoli florets harvested during either the winter or the early summer. Thus,
17	UV-B effectively delayed floret yellowing in various broccoli cultivars, harvested during
18	different seasons. Previously, UV-C and heat treatments have been applied to broccoli
19	florets; these treatments maintained Chl content and delayed floret yellowing. Moreover,
20	all of these treatments effectively inhibited Chl degradation enzyme activities, which are
21	involved in Chl breakdown (Funamoto et al., 2002; Costa et al., 2006). The delay of floret
22	yellowing by UV-B treatment may also suppress Chl-degrading enzyme activities.

1	In conclusion, the findings obtained in the present study show that UV-B treatment
2	delayed floret yellowing in broccoli. UV-A treatment did not similarly inhibit floret
3	yellowing. From cultivar to cultivar, the broccoli differed slightly in Chl content at harvest;
4	the 'Sawayutaka' cultivar exhibited higher Chl content than did the 'Pixel' cultivar. Chl
5	contents were also slightly higher in broccoli harvested during the winter season as
6	compared with the early summer season. However, UV-B doses of at least 8.8 kJ m^{-2}
7	effectively delayed the decrease in Chl content, suggesting that UV-B treatment will be
8	useful to maintain the postharvest quality of broccoli.
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2 Changes in hue angle value of broccoli florets with UV-A irradiation during storage at 15

3 °C.

UV-A treatment	Hue angle of surface color					
$(kJ m^{-2})$	Day 0	Day 0 Day 2		Day 6		
0	131.6 ± 0.60	132.8 ± 1.37	$131.0^{a}\pm0.31$	97.9 ± 1.58		
4.5	131.7 ± 2.00	132.4 ± 0.25	$128.8^{a}\pm0.40$	93.8 ± 1.10		
9.0	134.0 ± 2.30	132.3 ± 0.23	$126.0^b\pm0.74$	94.9 ± 0.74		
F-test	ns	ns	*	ns		

The results were expressed as means ± standard error for three broccoli florets in each
treatment. Different letters within same column indicate significant difference between
treatments. The asterisk (*) indicates that the value is significantly different from
corresponding control (*p* < 0.05). (ns) indicates that the value is not significantly different
from corresponding control.

2 Changes in hue angle value of broccoli florets with UV-B irradiation during storage at 15

3 °C.

UV-B treatment	Hue angle of surface color					
(kJ m ⁻²)	Day 0	Day 2	Day 4	Day 6		
0	132.3 ± 0.91	132.2 ± 1.24	$115.1^{c} \pm 0.88$	$94.7^{b} \pm 1.55$		
4.4	131.3 ± .096	133.4 ± 0.72	$115.9^{\rm c} \pm 1.19$	$92.8^b \pm 4.48$		
8.8	132.9 ± 0.78	131.2 ± 0.24	$121.7^b\pm0.60$	$107.7^{a} \pm 1.66$		
13.1	131.5 ± 0.42	132.1 ± 0.57	$122.0^b\pm0.95$	$107.0^{a} \pm 1.88$		
17.5	130.9 ± 0.48	132.1 ± 0.53	$122.6^{b} \pm 1.56$	$104.3^{a} \pm 1.11$		
26.3	129.9 ±0.34	130.1 ± 0.80	$126.2^{a}\pm0.59$	$108.0^{a}\pm0.93$		
F-test	ns	ns	*	*		

The results were expressed as means ± standard error for three broccoli florets in each
treatment. Different letters within same column indicate significant difference between
treatments. The asterisk (*) indicates that the value is significantly different from
corresponding control (*p* < 0.05). (ns) indicates that the value is not significantly different
from corresponding control.

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- 2 Changes in the hue angle value and total chlorophyll contents of two cultivars of broccoli
- 3 florets ('Pixel' and 'Sawayutaka') with or without UV-B (8.8 kJ m^{-2}) treatment during
- 4 storage at 15 °C.

Cultivora	UV-treatment (kJ m ⁻²)	Hue angle value of surface color			Total chlorophyll content (g kg ⁻¹ FW)				
Cultivals		Day 0	Day 2	Day 4	Day 6	Day 0	Day 2	Day 4	Day 6
Pixel	0	132.2	132.2	121.2 ^c	94.5 ^c	1.3	1.2 ^b	0.5 ^c	0.3 ^c
	8.8	132.3	131.2	129.7 ^b	112.4 ^b	1.3	1.2 ^b	0.8^{b}	0.6 ^b
Sawayutaka	0.0	133.9	132.9	129.3 ^b	97.2 ^c	1.3	1.3 ^a	0.8^{b}	0.4 ^{bc}
	8.8	134.8	133.2	132.5 ^a	129.7 ^a	1.3	1.3 ^a	1.1 ^a	0.8 ^a
F-test		ns	ns	**	**	ns	*	**	*

5 Different letters within column indicate significant difference between treatments and

6 cultivars. The asterisk (*) indicates that the value is significantly different from

7 corresponding control (p < 0.05). The asterisk (**) indicates that the value is significantly

8 different from corresponding control (p < 0.01). (ns) indicates that the value is not

9 significantly different from corresponding control.

10

2 Changes in the hue angle value and total chlorophyll contents of broccoli florets with or

3 without UV-B (8.8 kJ m⁻²) treatment during storage at 15 °C. The cultivar presented is

- 4 'Pixel', harvested in winter and early summer.
- 5

Harvest seasons	UV-treatment (kJ m ⁻²)	Hue angle value of surface color				Total chlorophyll content (g kg ⁻¹ FW)			
		Day 0	Day 2	Day 4	Day 6	Day 0	Day 2	Day 4	Day 6
Winter	0	132.2	132.2 ^a	121.2 ^a	94.5 ^b	1.2	1.1^{a}	0.7 ^b	0.3 ^b
	8.8	132.3	131.2 ^a	129.7 ^a	112.4 ^a	1.2	1.2 ^a	1.0^{a}	0.5 ^a
Early summer	0.0	135.9	125.3 ^b	104.2 ^b	94.5 ^b	1.2	0.6 ^c	0.4 ^c	0.2 ^c
	8.8	136.8	133.4 ^a	122.5 ^a	106.3 ^a	1.2	1.0 ^a	0.7 ^b	0.4 ^a
F-test		ns	*	*	*	ns	*	*	*

6 Different letters within same column indicate significant difference between treatments

7 and harvest seasons. The asterisk (*) indicates that the value is significantly different

8 from corresponding control (p < 0.05). (ns) indicates that the value is not significantly

9 different from corresponding control.

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