Pheophytinase activity and gene expression of chlorophyll-degrading enzymes relating to UV-B treatment in postharvest broccoli (*Brassica oleracea* L. Italica Group) florets

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Abstract

Pheophytinase (PPH) activity and gene expression of chlorophyll (Chl)-degrading enzymes relating to UV-B treatment in postharvest broccoli (*Brassica oleracea* L. Italica Group) florets were determined. PPH is involved in the dephytylation of Mg-free Chl *a*, pheophytin (Phy) *a*. However, chlorophyllase (Chlase, EC.3.1.1.14) also uses Phy *a* as a substrate to produce pheophorbide (Pheide) *a* by dephytylation. For an accurate determination of PPH activity, the PPH protein fraction was separated from Chlase protein by ammonium sulfate precipitation. The protein precipitated by 45-60% saturated ammonium sulfate was included a little bit Chlase activity and was suitable for PPH determination. PPH activity in broccoli florets treated with a UV-B dose of 19 kJ m^{-2} was repressed for the first 2 days of storage at 15 °C, whereas it increased gradually with senescence of control broccoli florets. The expression level of *BoCLH1* was reduced in broccoli florets on day 4 of storage, while *BoCLH2* and *BoCLH3* were up-regulated with UV-B treatment. A high *BoPAO* expression level was found in senescent broccoli florets, and the up-regulation of this gene was delayed by UV-B treatment. The highest expression level of *BoPPH* was found in the control, and its expression was clearly repressed by UV-B treatment on day 2 of storage. We suggest that the up-regulation of Chl-degrading enzyme genes could be delayed by UV-B treatment, resulting in the suppression of floret yellowing in stored broccoli.

Keywords: Chlorophyll degradation, Chlorophyllase, Pheophytinase, gene expression, UV-B treatment

1. Introduction

Plant senescence is often observed as a change in leaf color, which is regulated by chlorophyll (Chl) degradation. As shown in Fig. 1, the degradation of Chl to Chl catabolites occurs mainly in two ways, the removal of phytol from Chl *a* and the formation of chlorophyllide (Chlide) *a* by chlorophyllase (Chlase) (Roca and Minguez-Mosquera, 2003; Harpaz-Saad et al., 2007) followed by the removal of a Mg atom by a Mg-dechelating substance (MDS) (Shioi et al., 1996). Subsequently, pheophorbide (Pheide) *a* is degraded to fluorescent, primarily colorless Chl catabolites, including a red Chl catabolite generated by Pheide *a* oxygenase (PAO, EC 1.14.12.20) and a red Chl catabolite reductase (RCCR, EC 1.3.1.80) (Matile et al., 1999). An alternative process that differs in the first step of the above pathway proceeds by Chl *a* removing a Mg atom before the phytol group, with pheophytin (Phy) *a* as an intermediate (Tang et al., 2000, Vavilin and Vermaas, 2002). Afterward, the dephytylation of Phy *a* is catalyzed by Chlase (Heaton and Marangoni, 1996). Recently, pheophytinase (PPH) was also demonstrated to be a Phy *a*-specific dephytylation enzyme (Schelbert et al., 2009). Büchert et al. (2011b) found that the expression of *BoPPH* increased with floret yellowing of broccoli and that its expression was induced by ethylene; however, its expression was suppressed by cytokinin treatment. In addition, Chl *a* is also degraded in vitro by Chl-degrading 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* (Johnson-Flanagan and McLachlan, 1990; Kuroda et al., 1990; Martínez et al., 2001; Yamauchi et al., 2004). Pheophorbidase (PPD, EC 3.1.1.82) is involved in the Chl

degradation pathway, and it converts Pheide *a* to pyropheophorbide (PyroPheide) *a* (Shioi et al., 1996).

Broccoli is a kind of cruciferous vegetable with a high antioxidant content. However, broccoli is a highly perishable product and floret yellowing is a major limitation to extended shelf life with high quality. Physical treatments have been applied to delay floret yellowing, including heat treatments (Funamoto et al., 2002; Costa et al., 2006b; Kaewsuksang et al., 2007), low temperature storage (Starzyńska et al., 2003), controlled atmosphere storage (Yamauchi and Watada, 1998) and UV-C treatment (Costa et al., 2006a; Erkan et al, 2008). Previously, we discovered that UV-B treatment effectively inhibited Chl-degrading enzymes, such as Chlase, Chl-degrading POX and MDS, in stored broccoli (Aiamla-or et al., 2010). In the present study, we deal with gene expression of Chl-degrading enzymes, corresponding to the Chl degradation pathway, during storage of broccoli florets with postharvest UV-B treatment. Furthermore, we defined the methodology for PPH activity analysis and characterized the enzyme and corresponding changes in senescing broccoli florets.

2. Materials and Methods

2.1 Plant materials and UV-B treatments

Broccoli (*Brassica oleracea* L. Italica Group, cv. Sawayutaka) heads were harvested in 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) according to Aiamla-or et al. (2010). Each broccoli head was placed vertically under the UV-B lamps at a distance of 15 cm, resulting in UV-B energy doses of

19 kJ m^{-2}. The dose of UV-B treatment was detected by a UV-B meter (UV 6.0 MK Scientific), which measured rang of 285-315 nm. Broccoli heads 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 plant materials were removed at scheduled intervals during the 6-day storage period. The florets used for gene expression analysis were frozen with liquid nitrogen and kept at –80 °C. For determination of enzyme activities, florets were kept as acetone powders at –20 °C.

2.2 Chlorophyll assay and chlorophyll *a* and pheophytin *a* preparation

Chl content was determined using *N*,*N*-dimethylformamide (Moran, 1982). For Chl *a* preparation, spinach leaves were homogenized for 3 min in cold acetone (–20 °C). The homogenate was filtered through two layers of Miracloth (Calbiochem, USA). The filtrates were treated with dioxane and distilled water to precipitate a lipid-soluble pigments including Chls 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 in the dark. Afterwards, the soluble pellets were centrifuged at 10,000 × g for 15 min at 4 ºC and then subsequently dissolved in petroleum ether. Soluble chlorophyll in petroleum ether was flushed with nitrogen gas, then stored at –20 °C until the individual pigments were separated using sugar powder column chromatography (Perkins and Roberts, 1962). Finally, five hundred µg/mL of Chl *a* was prepared in acetone. Phy *a* was prepared by the acidic reaction, using 0.1 M hydrochloric acid (HCl). The Phy *a* concentration was measured spectrophotometrically at 409 nm using an extinction coefficient of 156,000 M cm^{-1}.

2.3 Protein extraction and separation

An acetone powder (1 g) of floral tissues was suspended in 15 mL of 50 mM Tris (hydroxymethyl) aminomethane-hydrochloric acid (Tris-HCl) buffer (pH 8). The mixture was stirred for 1 h at 0 °C and filtered with two layers of Miracloth. The filtrate was then centrifuged at 15,000× g at 4 °C for 15 min. The protein in the supernatant was precipitated with saturated ammonium sulfate. The precipitate was dissolved with 50 mM Tris-HCl buffer (pH 8) and the solution was passed through a PD-10 column (GE Healthcare) to desalt the ammonium sulfate. Using the eluate, the formation of Pheide *a* was measured by high performance liquid chromatography (HPLC) analysis, as previously described (Aiamla-or et al., 2010). Chlase and PPH activities were also determined spectrophotometrically at 667 nm, by measuring Pheide *a* or Chlide *a* formation. The protein contents were determined based on Bradford's method (1976).

2.4 Determination of chlorophyllase and pheophytinase activities

For the Chlase assay, the reaction mixture contained 0.75 mL of 20 mM phosphate buffer (pH 7.5), 0.1 mL of 500 μg/mL Chl *a* acetone solution and 0.5 mL of enzyme solution. The reaction mixture was incubated in a water bath at 25 °C for 40 min, and afterward 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*, whereas the lower phase contained the Chlide *a*. The activity was spectrophotometrically detected by Chlide *a* formation at 667 nm. The unit of enzyme activity, katal (kat), was defined as the amount of enzyme that formed one mole of product per second.

PPH activity was based on the method of Shchelbert et al. (2009), with the slight modification of performing the spectrophotometric measurement at 667 nm. The reaction mixture contained 0.35 mL of enzyme solution, 75 μL of 2.0% Triton X–100, 0.1 mL of Phy *a* (10 μM) and 0.70 mL of 50 mM Tris–HCl buffer (pH 8.0). The reaction was incubated in darkness for 90 min at 25 °C. After the optimal concentration of ammonium sulfate was defined for PPH protein as described above, the assay on optimal reaction incubation conditions for PPH activity was carried out. The partial PPH protein fraction, which was precipitated by 45-60% saturated ammonium sulfate, was used to determine enzyme activity. The reaction was stopped by addition of acetone. Pheide *a* was separated from Phy *a* by additional hexane. Subsequently, the Pheide *a* concentration in the acetone layer was measured spectrophotometrically at 667 nm. The quantity of Pheide *a* formation was calculated by comparison to a Pheide *a* standard (Wako Pure Chemical Industries, Tokyo, Japan). PPH activity was expressed as products of Pheide *a* (mole) per second, katal (kat) per mg protein.

2.5 RNA extraction and real time PCR analysis of transcriptional profile of chlorophyll-degrading enzymes

Frozen broccoli florets were ground in liquid nitrogen, and powder was put into 150 mM Tris-HCl buffer (pH 7.5) containing 5 mM ethylenediaminetetraacetic acid (EDTA), 2% sodium dodecyl (SDS) and 3.55 μM mercaptoethanol in a total volume of 5 mL. Total RNA was prepared by the phenol method and quantified by spectrophotometry. RNA integrity was determined on 2% agarose gels by electrophoresis. Total RNA was purified with phenol / chloroform / isoamyl alcohol (PCI) and precipitated by adding 1/3 extraction volume 10 M

LiCl overnight at –20 ºC. After RNA in LiCl solution was centrifuged at 10,000× g at 4 ºC for 10 min, the pellets were collected. Subsequently, pellets were re-dissolved in DEPC-DW. RNA was purified again with PCI and precipitated by adding 1/10 sample volume 3 M sodium acetate and 2.5 sample volume 99.5% ethanol at –80 ºC for 20 min. Twenty-five micrograms of RNA were treated with DNase. Ten micrograms of RNA was reverse transcribed using a cDNA synthesis kit (Takara). cDNA was stored at –20 ºC and employed as a template for two step RT–PCR using the SYBR® Green PCR Master Mix (QIAGEN) according to manufacturer recommendations. Primers were designed using Primer Express® software version 3.0 (Applied Biosystem). Actin was used as an internal control: *BoAct1* (accession no. AF044573), forward 5'–CTTGCACCAAGCAGCATGAA–3', reverse 5'–AGAATGGAACCACCGATCCA–3'. Primers specific to following genes were used: *BoCHL1* (accession no. AF337544), forward 5'–CGGTTTTGTCGGGTTTATGG–3', reverse 5'– CACCAACAAAGCTTCTCATCTCA–3'; *BoCLH2* (accession no. AF337545), forward 5'–TCTCGCTGTCGCTGCTACAA–3', reverse 5'–GTTGCCACCAAAAGCGACTT–3'; *BoCLH3* (accession no. AF337546), forward5'–GGTGATGCTCCTCCATGGTT–3', reverse 5'–TGAAGCCATGGGAAGAGACAT–3' and *BoPAO* (accession no. AB470926), forward 5'–ACTAGGATTCCACAGGCTGCTAC–3', reverse 5'–CCATTTTCATCAGGCCACAC–3'. Primer specific to *BoPPH* was followed Buchert et al. (2011b)`s designed; forward 5'–AGAGGTTATCGGTGAGCCA–3', reverse 5'–GACGAGATGAGGATGGG–3'. Each measurement was performed in triplicate.

3. Results

3.1 Accurate determination of pheophytinase activity using saturated ammonium sulfate precipitation

In the Chl degradation pathway, Chlase normally utilizes both Chl *a* and Phy *a* as substrates to produce Chlide *a* and Pheide *a*, respectively (McFeeters et al., 1971; Mínguez-Mosquera et al., 1994), whereas PPH has specific activity only for Phy *a*. A high level of Pheide *a* formation was found in reactions with the 20-40% and 40-60% saturated ammonium sulfate fractions, while a high level of Chlide *a* formation occurred in reaction with the 20-40% saturated ammonium sulfate fraction (data not shown). These results indicated that both PPH and Chlase proteins were present in the 20-60% saturated ammonium sulfate precipitate. Hence, we further separated PPH protein from Chlase protein using 0-40%, 40-50%, 50-60%, 0-45% and 45-60% saturated ammonium sulfate fractionations. As shown in Fig. 2, Chlase protein was highly precipitated with 0-40% saturated ammonium sulfate, whereas the formation of Pheide *a* was found in each fraction over a wide range of the 0–60% saturated ammonium sulfate. By further separation using 0–45% and 45–60% saturated ammonium sulfate precipitation, most of the protein was precipitated with the 0–45% fraction. Chlase activity was also present in this fraction, with greater specific activity in the 0-45% fraction than in the 0-40% fraction (Fig. 2C). Little Chlase activity was found in the 45-60% fraction. On the other hand, Pheide a formation was found in both the 0-45% and 45-60% fractions (Fig. 2D), indicating that Chlase protein was present in the 20-45% fraction and PPH protein in the 45-60% fraction. Based on these results, the 45–60% fraction was used to characterize PPH and its activity changes in stored broccoli florets.

3.2 Characterization of pheophytinase

Enzyme characterization was performed on the partially purified PPH. As apparent in Table 1, K*m* values corresponding to Chl *a* and Phy *a* of Chlase were approximated to be 14.54 μM and 18.18 μM, respectively. Chlase on Chl *a* also showed a high V*max*/K*m* value (194.09) but a low value of V*max*/K*m* (0.43) on Phy *a*. The K*m* and V*max*/K*m* values of PPH on Phy *a* were estimated to be 15.89 μM and 0.49, respectively. The pH optimum for PPH activity was around 8 and its optimum temperature was 25 ºC (data not shown).

3.3 Pheophytinase activity changes during storage in broccoli florets after UV-B treatment

Chls *a* and *b* contents in the control showed a slight decrease for the first 2 days of storage at 15 °C and then decreased rapidly concomitant with floret yellowing (Fig. 3). UV-B treatment suppressed the decline in Chl level during storage and delayed the progress of floret yellowing by around 2 days. In fresh broccoli florets, PPH activity was approximately 0.6 *p*kat/mg protein in controls, while it was 0.5 *p*kat/mg protein florets treated with UV-B. PPH activities in broccoli florets with or without UV-B treatment showed a gradual increase during storage at 15 ºC. UV-B treatment did not significantly affect PPH activity in stored broccoli florets (Fig 3).

3.4 Effects of UV-B treatment on gene expression levels of chlorophyll-degrading enzymes

Changes in gene expression of enzymes involved in Chl degradation were determined by real time PCR (qRT-PCR) analysis using specific primers from *B. oleracea*. The results of gene expression by qRT-PCR are shown in Fig 4. The expression of all Chlase genes (*BoCLH1*, *BoCLH2* and *BoCLH3*) increased immediately after UV-B treatment, whereas the expression of *BoPPH* showed a small reduction with UV-B treatment (Figs. 4A, 4B and 4C). The expression level of *BoCLH1* in UV-B treated broccoli florets was reduced on day 4 in comparison with that of the control. However, UV-B treatment did not reduce the expression level of *BoCLH2* or *BoCLH3* during storage. The expression of Chlase genes was reduced with senescence of control florets. The highest expression level of *BoPAO* in control broccoli was found on day 4 and that in UV-B treated broccoli on day 6 of storage. UV-B treatment reduced the expression level of *BoPAO* in broccoli florets on days 2 and 4 (Fig. 4D) relative to controls. Likewise, the highest expression level of *BoPPH* was found in the control, and its expression was clearly repressed by UV-B treatment on day 2. The highest expression level of *BoPPH* in broccoli with UV-B treatment was shown on day 4, but the level was similar to that of the control on day 4 (Fig. 4E).

4. Discussion

When broccoli heads are harvested, their florets are immature and in their most intense growth phase, which makes them very sensitive to stress factors, leading to a rapid initiation of senescence. Therefore, suitable treatments are required to delay degradation of Chl in broccoli florets during storage. Several techniques have been applied to maintain the green color of broccoli florets (Funamoto et al., 2002; Costa et al., 2005; Costa et al., 2006a; Costa

et al., 2006b). We previously applied UV-B doses of 19 kJ m^{-2} to broccoli florets. We found that a UV-B dose of at least 19 kJ m^{-2} effectively delayed the progress of floret yellowing and Chl degradation. Furthermore, we also reported that the activity levels of Chl-degrading enzymes such as Chlase, Chl-degrading POX and MDS were reduced by UV-B treatment in stored broccoli florets (Aiamla-or et al., 2011). Recently, PPH was reported to be an important enzyme involved in Chl degradation, such that *Arabidopsis* mutants deficient in PPH accumulated Phy *a* during senescence (Shcellbert et al., 2009). However, the accurate measurement of Pheide *a* formation by PPH during plant senescence could be difficult because Chlase also uses Phy *a* as a substrate. Büchert et al. (2011b) reported that the expression level of *PPH* increased with the progress of senescence in broccoli florets, but changes in PPH activity was still unknown. Hence, we defined a methodology for measuring PPH activity in broccoli florets using Phy *a* as a substrate. Saturated ammonium sulfate was used for protein precipitation, and Pheide *a* formation was found in the 20-40% to 40-60% fractions, while Chlide *a* formation was found in the 20-40% fraction (data not shown). The formation of Pheide *a* as well as Chlide *a* was high in the 0-40% and 0-45% fractions. Therefore, the fractions precipitated with 0-40% or 0-45 % saturated ammonium sulfate seemed to include Chlase, which uses both Chl *a* and Phy *a* as substrates. This characteristic of Chlase has been verified using recombinant protein, which revealed enzyme activity with both Chl *a* and Phy *a* (Okazawa et al., 2006). The protein fraction precipitated with 45-60 % saturated ammonium sulfate hardly showed Chlide *a* formation by Chlase, but did exhibit a high level of Pheide *a* formation. Taking these results together, we suggested that the protein fraction precipitated with 45-60% saturated ammonium sulfate should be suitable for PPH determination. Moreover, K*m* and V*max*/K*m* values corresponding to Phy *a* of Chlase were

not significantly different from those values of PPH, indicating that both PPH and Chlase have almost the same substrate affinity and catalytic power corresponding to Phy *a*.

In stored broccoli florets, PPH activity increased concomitantly with the decline of Chls *a* and *b* contents, which suggests that PPH could be involved in Chl degradation in stored broccoli florets. UV-B treatment effectively suppressed Chl degradation with floret yellowing during storage. On the other hand, the enhancement of PPH activity in UV-B-treated broccoli florets was slightly reduced for the first 2 days of storage; afterward its activity increased in common with changes in PPH activity in the control. These findings indicate that the inhibitory effect of Chl degradation by UV-B treatment might be due to the suppression of enzymatic reactions except that of PPH. As discussed in a previous paper (Aiamla-or et al., 2010), MDS may possibly play a crucial role in Chl degradation of broccoli florets, but as the molecular nature of MDS and the involvement in MDS during Chl degradation are not clearly understood, a future study is needed to elucidate this point.

The relative expression levels of *BoCLH1*, *BoCLH2*, *BoCLH3*, *BoPAO* and *BoPPH* genes in broccoli florets with or without UV-B treatment were determined by qRT-PCR. The expression level of *BoPPH*, as well as PPH activity, was reduced immediately after UV-B treatment. The highest expression level of *BoPPH* in the control was found on day 2 of storage. UV-B treatment considerably repressed expression on day 2, but then the expression level of *BoPPH* increased in UV-B-treated broccoli florets during storage. Repression similar to that of UV-B treatment was found with heat, UV-C and cytokinin treatments, whereas modified atmosphere storage did not repress the expression level of *BoPPH* in stored broccoli florets (Büchert et al., 2011ab). UV-B treatment, however, did not reduce the expression level of *BoCLH2* or *BoCLH3* in stored broccoli florets. In addition, *BoCLH2* in broccoli

florets with UV-B treatment showed an irregular transcriptional profile during storage, the same as that found with cytokinin treatment (Büchert et al., 2011b). It was suggested that both *BoCLH2* and *BoCLH3* could be unimportant in Chl degradation of broccoli florets because their expression patterns did not correlate with degradation of Chl (Büchert et al., 2011) and were not detected in florets of *B. oleracea* var. *italica* (Chen et al., 2008). In this study, mRNA transcripts of all the Chlase genes, *BoCLH1*, *BoCLH2* and *BoCLH3*, were found in broccoli florets (*B.oleracea* cv. pixel).

In the Chl-degradation pathway with conversion of Pheide *a* to pFCC, PAO plays a significant role. PAO was considered to be an important enzyme in Chl degradation, because its activity was found only during senescence (Chung et al., 2006; Pružinská et al., 2003). The highest expression level of *BoPAO* was found in the control on day 4 and in UV-B-treated broccoli on day 6 of storage. UV-B treatment reduced the expression level of *BoPAO* in broccoli florets on days 2 and 4. Fukasawa et al. (2009) reported that ethanol vapor treatment suppressed Chl degradation in broccoli flortes during storage and also reduced the expression level of *BoPAO*. Hence, the control of PAO by postharvest treatment could be important for suppression of senescence.

In conclusion, an accurate, simple assay for PPH activity determination has become possible using ammonium sulfate precipitation. PPH activity and *BoPPH* gene expression was reduced by UV-B treatment of 19 kJ m^{-2} for the first 2 days of storage, but then they increased with senescence in broccoli florets with or without UV-B treatment. A high *BoPAO* expression level was found in senescent broccoli florets, and up-regulation of its expression was delayed by UV-B treatment. Therefore, UV-B treatment reduced gene expression of Chl-degrading enzymes, resulting in retarded Chl degradation in broccoli florets during storage.

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Figure captions;

Fig. 1. Putative pathway of chlorophyll degradation

Fig. 2. Protein fractions and formation of Chlide *a* and Pheide *a* by ammonium sulfate precipitation. Each fraction was precipitated by 0–40%, 40–50%, or 50–60% as a first separation and 0–45% and 45–60% as a second separation (A and B). The formations of Pheide *a* by Chlase and PPH and Chlide *a* by Chlase: A, total Chlide *a* formation: B, total Pheide *a* formation: C, Chlide *a* formation per mg protein: D Pheide *a* formation per mg protein. Vertical bars represent average values with ± SE (n = 3)

Fig. 3. Changes of PPH activity, Chl *a* and *b* contents in broccoli florets with or without UV-B treatment (19 kJ m^{-2}) during storage at 15 ºC. Vertical bars represent average values with ± SE (n = 3).

Fig. 4. Relative expression as measured by qRT-PCR of *BoCLH1* (A), *BoCLH2* (B), *BoCLH3* (C), *BoPAO* (D) and *BoPPH* (E) in broccoli florets with or without UV-B treatment (19 kJ m^{-2}). Vertical bars represent average values with ± SE (n = 3).

Table 1. Kinetic constants corresponding to Chl *a* and Phy *a* of PPH and Chlase.

Properties	Pheophytinase	Chlorophyllase	
	Phy *a*	Chl *a*	Phy *a*
K*m* (μM)	15.89	14.54	18.18
V*max* (*p*kat)	7.82	2826	7.69
V*max*/K*m*	0.49	194.09	0.43

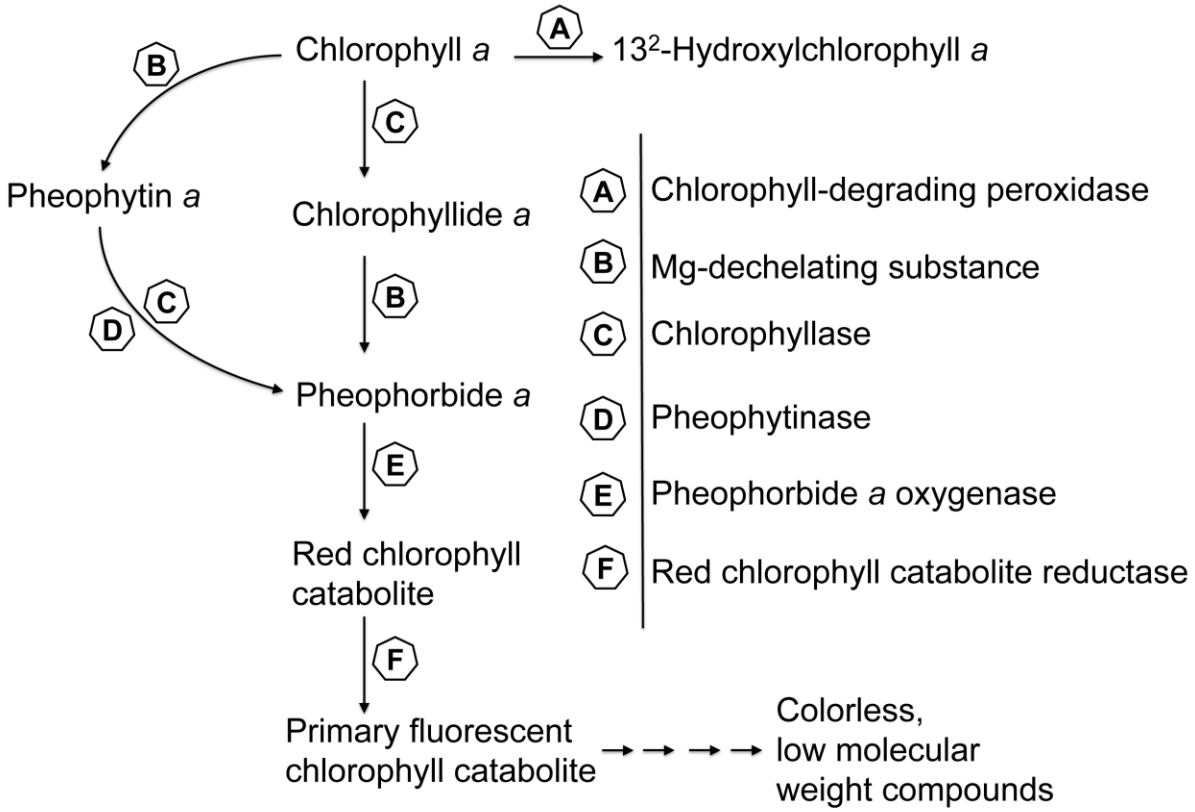
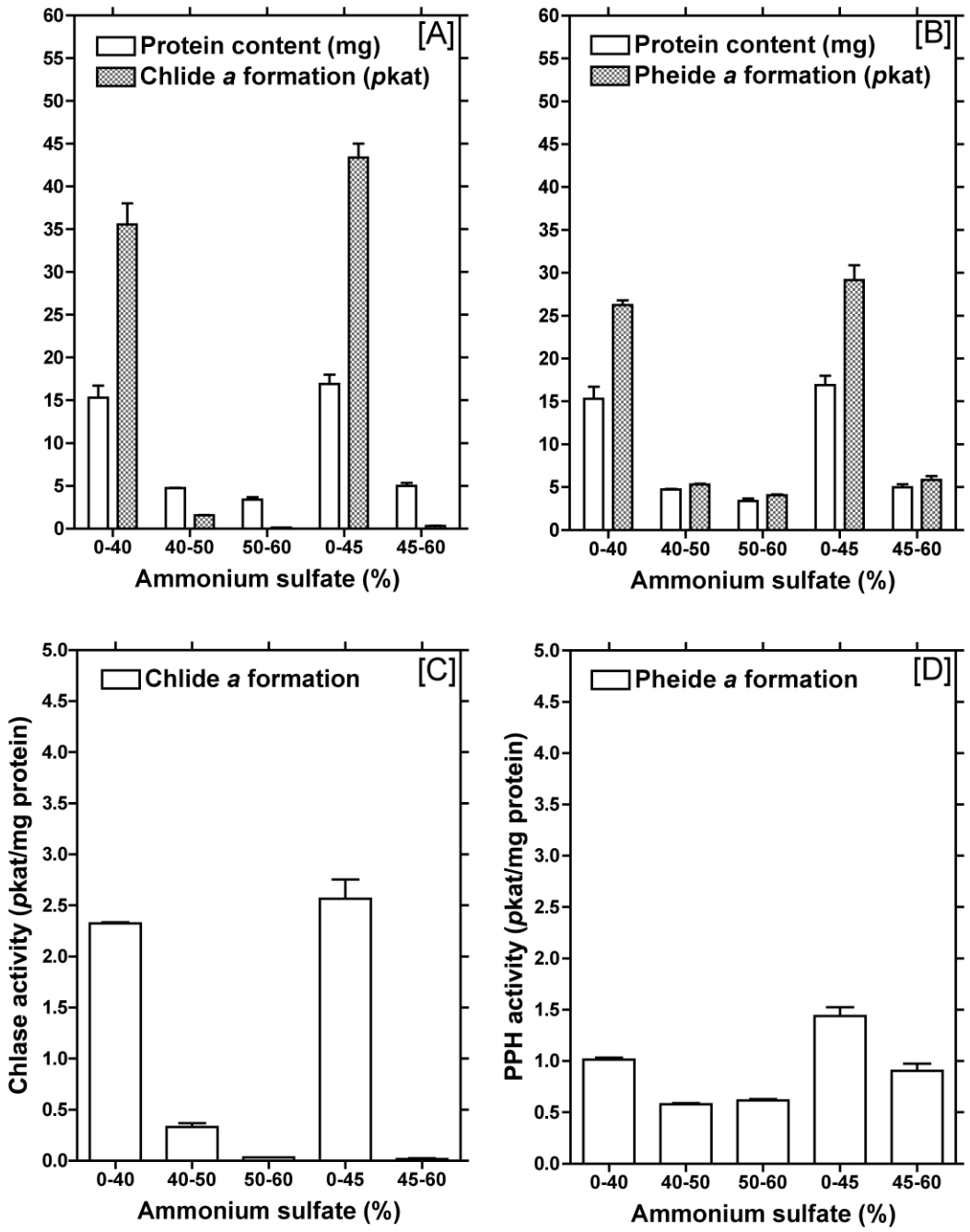

Chlorophyll a
A
13²-Hydroxylchlorophyll a
B
C
Pheophytin a
Chlorophyllide a
A Chlorophyll-degrading peroxidase
B Mg-dechelating substance
C Chlorophyllase
D Pheophytinase
E Pheophorbide a oxygenase
F Red chlorophyll catabolite reductase
B
C
D
Pheophorbide a
E
Red chlorophyll catabolite
F
Primary fluorescent chlorophyll catabolite
Colorless, low molecular weight compounds

Fig. 1


[A]
Protein content (mg)
Chlide a formation (pkat)
Ammonium sulfate (%)
0-40
40-50
50-60
0-45
45-60
[B]
Protein content (mg)
Pheide a formation (pkat)
Ammonium sulfate (%)
[C]
Chlide a formation
Chlase activity (pkat/mg protein)
Ammonium sulfate (%)
[D]
Pheide a formation
PPH activity (pkat/mg protein)
Ammonium sulfate (%)

1
2 Fig. 2

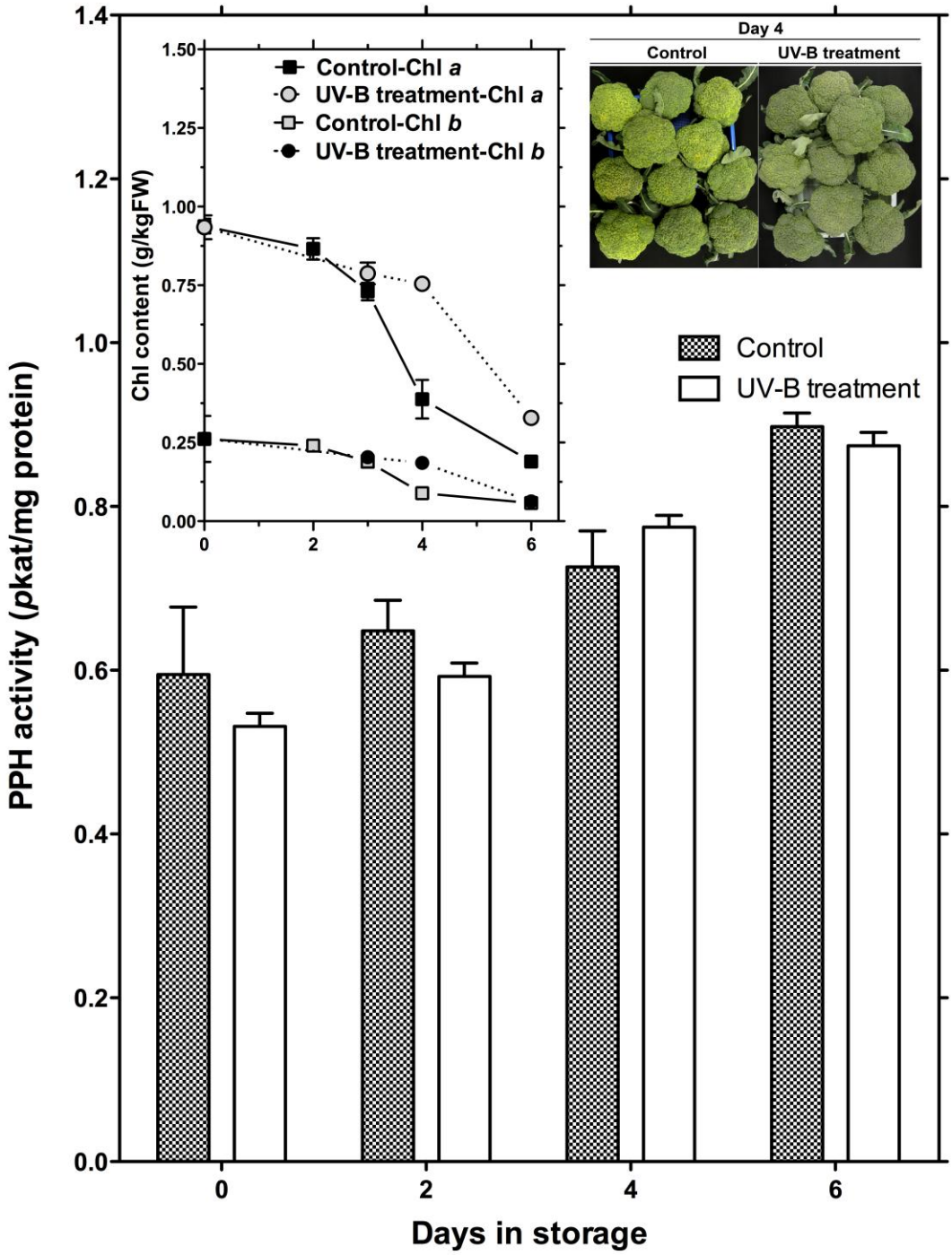

PPH activity (pkat/mg protein)
Days in storage
Control
UV-B treatment
Chl content (g/kgFW)
Control-Chl a
UV-B treatment-Chl a
Control-Chl b
UV-B treatment-Chl b
Day 4
Control
UV-B treatment

Fig. 3

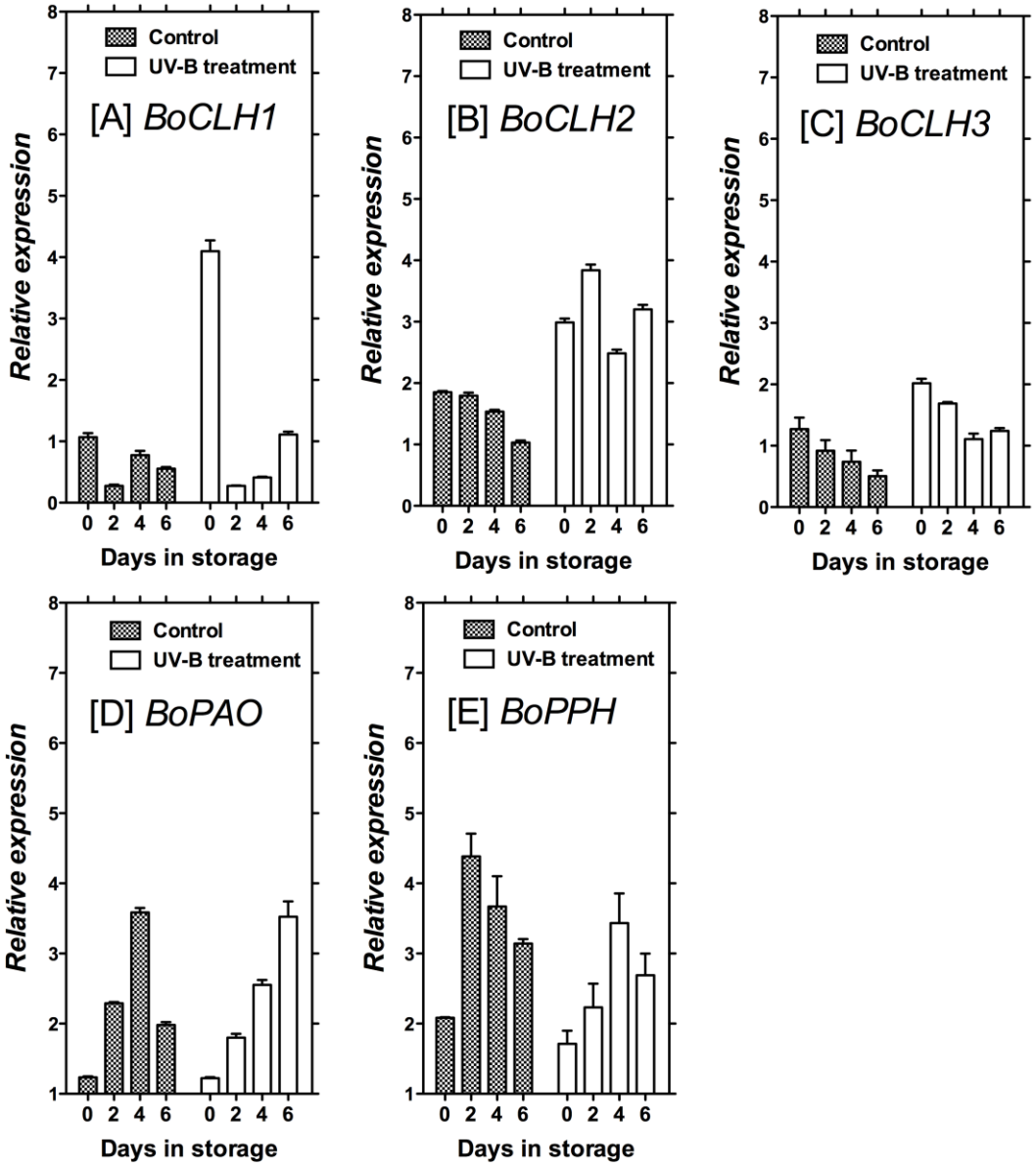

Control
UV-B treatment
[A] BoCLH1
[B] BoCLH2
[C] BoCLH3
[D] BoPAO
[E] BoPPH
Relative expression
Days in storage
0 2 4 6 0 2 4 6

Fig. 4