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6-Benzylaminopurine Delays Senescence and Enhances Health-Promoting Compounds of Harvested Broccoli

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ABSTRACT: The effect of 6-benzylaminopurine (6-BA) on the color, antioxidant activity, and contents of total phenols, glucosinolate, and sulforaphane in broccoli florets was investigated. The results showed that 6-BA treatment markedly inhibited the increase of the L^* value and malondialdehyde (MDA) content and retarded the decrease of the H value. 6-BA treatment reduced the rate of chlorophyll degradation by regulating the activities of chlorophyllase and Mg-dechelatase. When compared to control florets, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity and the activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) were enhanced in florets treated with 6-BA, whereas the activity of peroxidase (POD) was significantly reduced. The contents of total phenols, glucosinolate, and sulforaphane in broccoli florets were also profoundly increased after treatment with 6-BA. These results indicated that 6-BA could maintain the quality, delay senescence, and improve the nutritional value of broccoli.

KEYWORDS: *broccoli, 6-benzylaminopurine (6-BA), antioxidant enzymes, senescence, glucosinolate, sulforaphane*

INTRODUCTION

Broccoli (*Brassica oleracea* L. ssp. *italica*) is known to have high nutritional value with significant contents of vitamins, antioxidants, and health-promoting compounds such as glucosinolates and phenolic compounds.[1,2] However, broccoli deteriorates rapidly, and the florets turn yellow at room temperature after harvest. Harvesting and the following processing cause severe stress, hastening the appearance of senescence symptoms.[3] The effect of different storage methods on extending shelf life and improving visual quality and nutritional quality of broccoli has been widely investigated.[4,5]

Glucosinolates as an important class of secondary metabolites have gained particular interest as potential anticancer and chemopreventing agents. Glucosinolates are chemically stable until they come in contact with the enzyme myrosinase, which is compartmentalized from glucosinolates in plant tissue.[6] Sulforaphane is a naturally occurring sulfur-containing isothiocyanate, which is formed from its glucosinolate (glucoraphanin) by myrosinase when broccoli tissue is crushed or chewed. Many epidemiological studies have shown that sulforaphane has antitumor activity, inhibiting cell growth and stimulating apoptosis in cultured cancer cells[7] and decreasing the rate of tumor growth in animals with chemically induced cancers.[8] Although investigations of the effects of storage and cooking on the glucosinolate content of broccoli have been performed,[9] little information is available regarding the effects of storage or cooking on the formation of sulforaphane in broccoli.[10,11]

In intact plants, cytokinins are synthesized in the roots and transported to the flowers, and after harvest their concentration decreases abruptly.[12] Cytokinins including kinetin and 6-benzylaminopurine (6-BA) have been considered nontoxic for human and the environment as plant growth regulators by the U.S. Environment Protection Agency (EPA). 6-BA is regarded as a good candidate for postharvest applications and approved as a biopesticide in organic produce in the United States and Canada.[13] The cytokinin group of plant hormones may play an important role in controlling senescence in leaves, because a decline in cytokinin concentrations occurs in senescing leaves and external application of cytokinin also often delays leaf senescence.[14] Dipping broccoli florets in 6-BA depressed the respiration rate and delayed floret yellowing.[15] Downs et al.[14] found that cytokinin treatment can alter the physiological changes which usually accompany the senescence of broccoli florets. However, limited information is available on the effect of 6-BA treatment on antioxidant enzymes, glucosinolates, and sulforaphane in broccoli florets. Our objectives were to examine the effect of 6-BA treatment on antioxidant enzyme activities (superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX)), and health-promoting compounds (glucosinolates and sulforaphane) along with chlorophyll contents, chlorophyllase and Mg-dechelatase activities, and visual quality in broccoli florets during storage at 15 °C.

MATERIALS AND METHODS

Plant Material and Treatment. Broccoli (*B. oleracea* L. var. *italica* cv. Chaoda No. 1) heads were obtained from local producers (Nanjing, province of Jiangsu, China). The heads were placed on ice and returned to the laboratory within 6 h of harvest. Broccoli heads were divided into two groups. The whole broccoli heads in the first group were dipped in 200 mg/L 6-BA (this concentration was chosen as optimal for extending the shelf life of broccoli on the basis of our previous study) for 1 min at room temperature, and those in the second group were dipped in distilled water as control. They were then dried on absorbent paper and stored in an incubation chamber

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with 95% relative humidity at 15 °C. Triplicate samples were taken, and a total of 30 heads of broccoli were used in the experiment. Samples were taken daily, and florets were removed from the stems. Then the florets were immediately frozen in liquid nitrogen and kept at −20 °C for later analysis.

Color Measurement. Floret color was determined on intact heads of broccoli using a Minolta Chromameter (CR 400). Throughout the experiment, color readings were evaluated at five positions on each head daily. The hue angle was calculated as $h^0 = \tan^{-1}(b/a)$ when $a > 0$ and $b > 0$ or $h^0 = 180° + \tan^{-1}(b/a)$ when $a < 0$ and $b > 0$. L^* was lightness (0−100: 0 = black, 100 = white).

Determination of Chlorophyll Concentration and Chlorophyll-Degrading Enzymes. Chlorophyll concentrations were determined as described by Lichtentaler[16] with slight modifications. A sample of 0.16 g of florets was ground and extracted in 10 mL of 95% ethanol and then was centrifuged at 12000*g* for 10 min at 4 °C. The supernatant was used to determine the chlorophyll content. Chlorophyll quantification was performed spectrophotometrically at 665 and 649 nm, and the chlorophyll content was expressed as chlorophyll mass on a fresh weight basis (mg/g).

Chlorophyllase and Mg-dechelatase were extracted according to the methods of Funamoto et al.[17] An acetone powder (1 g) of floral tissues was suspended in 5 mL of 5 mmol L^{-1} phosphate buffer (pH 7.0) containing 50 mM KCl and 0.24% Triton X-100. The crude enzyme extracts were stirred for 0.5 h at 30 °C and centrifuged at 12000*g* for 20 min at 4 °C. The supernatants were used to determine chlorophyllase and Mg-dechelatase.

Chlorophyllase activity was determined according to a modified method of Amir-Shapira et al.[18] The reaction mixture contained 0.5 mL of enzyme solution, 0.2 mL of 1.44% Triton X-100, 0.2 mL of chlorophyll *a* acetone solution (100 μg/mL), and 0.5 mL of 0.1 M phosphate buffer (pH 7.5). The mixture was incubated in darkness at 30 °C for 45 min, and the enzyme reaction was stopped by the addition of 4 mL of acetone. The remaining chlorophylls were extracted with 4 mL of hexane and assayed by reading the absorbance at 663 nm. Activity was expressed as the increment of optical density at 663 nm per hour under the test conditions.

Mg-dechelatase was determined accordig to a modified method of Suzuki et al.[19] The reaction mixture contained 0.5 mL of 50 mM Tris-tricine buffer (pH 8.0), 0.7 mL of 100 μM chlorophyllin *a*, 0.4 mL of 1% Triton X-100, and 0.5 mL of a crude enzyme solution. The mixture was incubated at 30 °C for 30 min, and the activity was measured by following the change in OD at 686 nm. Mg-dechelatase activity was expressed as the increment of OD at 686 nm per hour under the test conditions.

Enzyme Assay. Broccoli florets (0.5 g) were homogenized with 5 mL of 50 mM phosphate buffer (pH 7.8) in an ice−water bath. The homogenates was centrifuged at 12000*g* for 10 min at 4 °C. The supernatant was collected for determination of the antioxidant enzyme activities.

Total superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), and peroxidase (POD, EC 1.11.1.7) activities were assayed according to the method of Toivonen et al.[20] One unit of SOD was the amount of extract that gives 50% inhibition of the reduction rate of NBT. Three milliliters of the reaction mixture contained 0.05 M phosphate buffer (pH 7.8), 13 mM methionine, 63 μM NBT, 1.3 μM riboflavin, and 0.1 mL of crude enzyme extract for extract or 0.1 mL of 0.05 mM phosphate buffer (pH 7.8) for control. CAT activity was determined by monitoring the enzyme-catalyzed decomposition of H_2O_2 by potassium permanganate. The reaction mixture contained 2 mL of 50 mM sodium phosphate buffer (pH 7.0), 0.5 mL of double-distilled water, and 0.5 mL of tissue extract, and the reaction was initiated by adding 2 mL of 30% (w/v) H_2O_2. POD activity was measured on the basis of the determination of guaiacol oxidation at 470 nm by H_2O_2. The reaction was carried out at 25 °C for 1 min in 3 mL of reaction mixture containing 0.038% (v/v) 30% H_2O_2, 100 mM sodium phosphate buffer (pH 6.0), and 0.056% (v/v) guaiacol.

Ascorbate peroxidase (APX, EC 1.11.1.11) activity measurement was adapted from the method of Vicente et al.[21] One unit of APX enzyme activity was defined as the amount of enzyme that produced an OD_{290} reduction per minute under the assay conditions.

Protein content in the enzyme extracts was determined according to the Bradford[22] method, using bovine serum albumin as a standard. The SOD, CAT, APX, and POD activities were expressed as units per milligram of protein.

Malondialdehyde (MDA) Determination. MDA was measured by 2-thiobarbituric acid reaction. One gram of frozen tissue was homogenized with 5 mL of 5% trichloroacetic acid (TCA) and then centrifuged at 12000*g* for 10 min at 4 °C. The supernatants were used to determine MDA content according to the method of Yang et al.,[23] and the amount of MDA was estimated as millimolar per gram FW.

DPPH Radical-Scavenging Activity Assay. The DPPH radical-scavenging activity was estimated following the method of Larrauri et al.[24] Half a gram of frozen sample was extracted with 50% ethanol and centrifuged at 12000*g* for 10 min at 4 °C. An ethanolic solution of DPPH served as control. The result was calculated according to the following formula: DPPH radical scavenging activity (%) = 1 − (absorbance of sample/absorbance of control) × 100%.

Total Phenol Content Assay. Frozen broccoli sample (0.5 g) was extracted with 80% acetone containing 0.2% formic acid, and the mixture was centrifuged at 12000*g* for 10 min at 4 °C. A sample of the crude extract (20 μL) was added to 180 μL of water and 1 mL of Folin−Ciocalteu reagent; 0.8 mL of a solution of 75 g/L Na_2CO_3 was added. The reaction mixture was incubated for 1 h at 30 °C. The absorbance was measured at 765 nm, gallic acid was used as a standard, and the results were expressed as milligrams of gallic acid per gram of tissue.

Determination of Total Glucosinolate Content. Total glucosinolate content was measured by using the method of Heaney et al.[25] with sight modifications. The method was based on the measurement of enzymically released glucose, which was hydrolyzed by the enzyme myrosinase (thioglcose glycohydrolase, EC 3.2.3.1). The content of glucose was determined by the method of phenol−sulfuric acid, to assay the absorbance at 490 nm, and then the amount of glucosinolate can be calculated from the glucose content.

Quantification of Sulforaphane. Sulforaphane was extracted from broccoli sample according to the method of Liang et al.[26] with minor modification. A sample of 0.2 g of freshly harvested broccoli was first milled using a mill grinder in the presence of liquid nitrogen and mixed with 2 mL 0.5 unit/mL myrosinase solution. After recovery of the liquid, the residue was dissolved in 1 mL of acetonitrile and was then filtered through a 0.45 μm membrane filter prior to injection into HPLC. Samples (20 μL) were separated at 30 °C on a Kromasil C18 column (250 × 4.6 mm i.d.; 5 μm particle size) using acetonitrile and water at a flow rate of 1.0 mL/min. The procedure employed isocratic elution with 20% acetonitrile for the first 10 min, then changed linearly over 13 min to 60% acetonitrile, and was maintained at 100% acetonitrile for 2 min to purge the column. Absorbance was measured at 254 nm.

Statistical Analyses. All data were expressed as the mean ± standard error (SE) and subjected to statistical analysis with the SPSS package program version 16.0 (SPSS Inc., Chicago, IL). The data were analyzed by one-way analysis of variance (ANOVA). Mean separations were performed by Duncan's multiple-range tests, and the differences at $p < 0.05$ were considered to be significant.

RESULTS

Effect of 6-BA Treatment on Visual Changes and Surface Color in Broccoli Florets. Changes of external color during broccoli senescence were evaluated through the hue angle (H) and L^* parameters. Control florets showed gradual reduction in their H value in the first 3 days of storage, but a drastic decrease on the fourth day, whereas the corresponding rate of change of 6-BA-treated florets was nearly 0 (Figure 1A). The decrease in the H value correlated with the progressive yellowing observed in the florets as the storage progressed.

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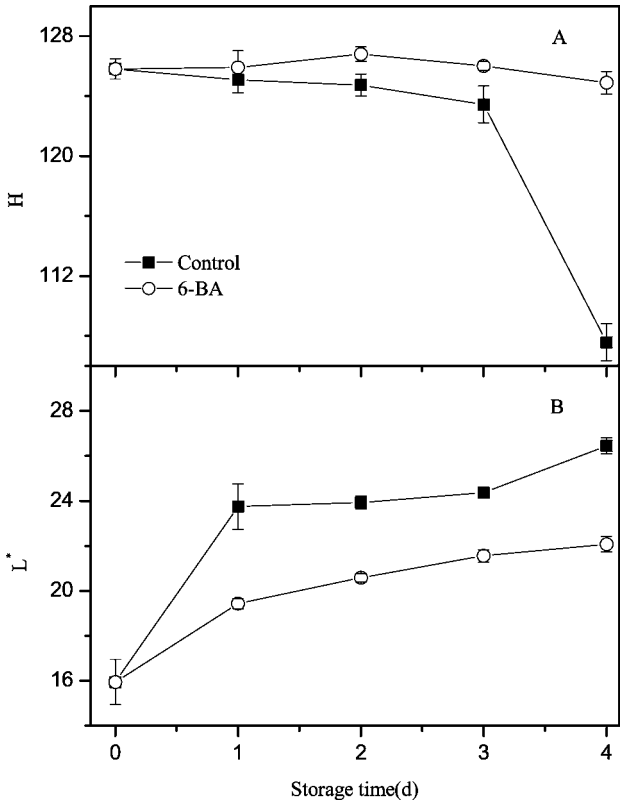

Figure 1. Changes of superficial color parameters (A, *H*; B, *L**) during postharvest senescence of broccoli incubated at 15 °C. Florets were treated with distilled water (control) and 200 mg/L 6-BA for 1 min at 20 °C. Each data point is the mean of three replicate samples. Vertical bars represent standard errors of the means.

The *L** value in both the control and treatment increased during storage (Figure 1B). However, the rate of increase of *L** value in 6-BA-treated florets was significantly slower than that of control florets.

Effect of 6-BA Treatment on Chlorophyll Content and Activities of Chlorophyll Catabolic Enzymes. The total chlorophyll content in all samples decreased continuously during storage (Figure 2A). Treatment with 6-BA delayed the loss of chlorophyll, and the treated florets maintained considerably higher chlorophyll content in comparison with the controls.

Chlorophyllase activity in florets of the control broccoli decreased gradually during the first 2 days in storage and then increased (Figure 2B). Chlorophyllase activity in florets of the 6-BA-treated broccoli showed a lower level than that in control florets and continued to decline throughout the postharvest period.

The activity of Mg-dechelatase increased along the course of senescence in both control and 6-BA-treated florets (Figure 2C). Similar to chlorophyllase, the activity of Mg-dechelatase in the 6-BA-treated broccoli showed a lower level than that in the control during the 4 days of storage.

Effect of 6-BA Treatment on MDA Content. The content of MDA in both control and 6-BA-treated broccoli florets increased as the storage progressed (Figure 3). 6-BA treatment profoundly inhibited the rise of MDA content, and the florets treated with 6-BA possessed relatively lower MDA content in comparison with the control florets during storage.

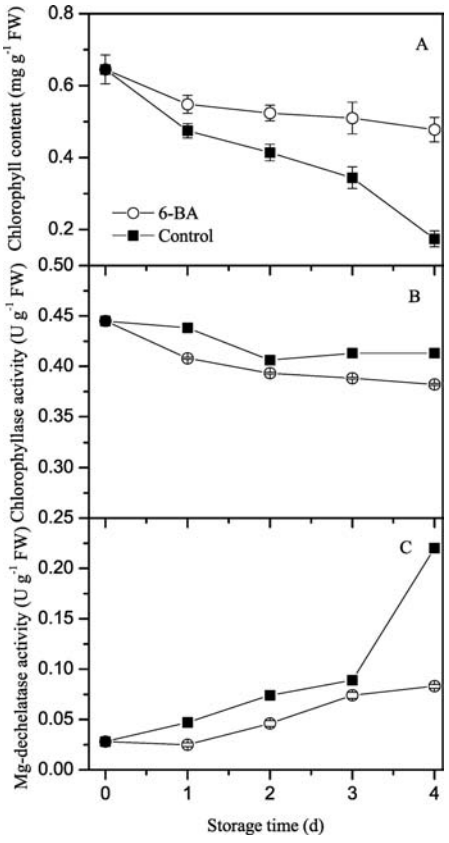

Figure 2. Changes of chlorophyll content (A) and chlorophyllase (B) and Mg-dechelatase activities (C) in broccoli florets after harvest. Data are shown for control and tissues treated with 200 mg/L 6-BA. Bars represent the standard errors of the means of triplicate assays.

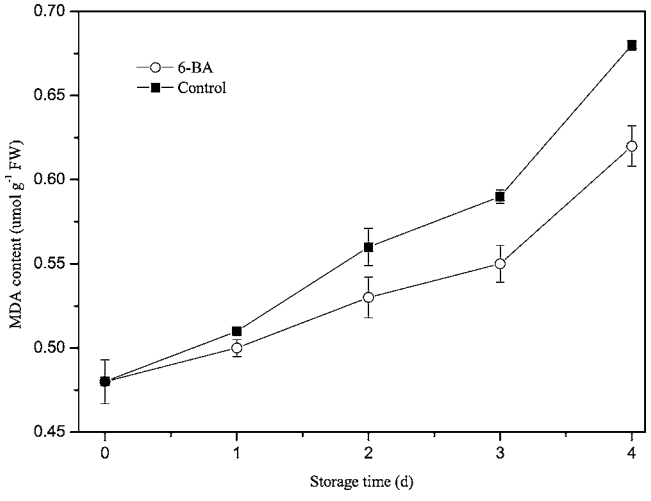

Figure 3. Change of malondialdehyde (MDA) content in broccoli florets after harvest. Data are shown for control and tissues treated with 200 mg/L 6-BA. Bars represent the standard errors of the means of triplicate assays.

Effect of 6-BA Treatment on CAT, APX, POD, and SOD Activities in Broccoli Florets. The activity of CAT in both the control and treated samples first decreased and then increased at 3 days of storage (Figure 4A). However, the florets treated with 6-BA possessed relatively higher CAT activity in comparison with the control florets during storage. APX activity in florets of the control broccoli tended to decline gradually

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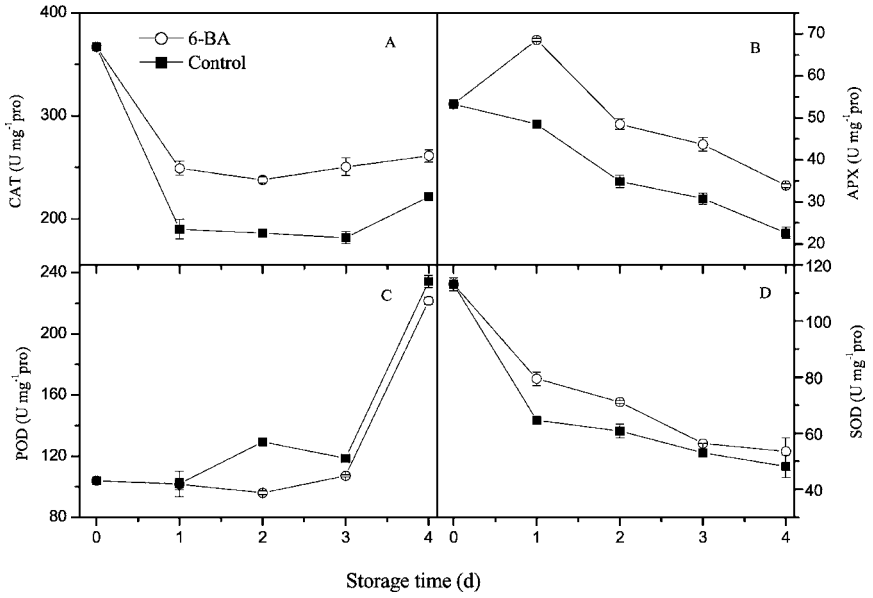

Figure 4. Changes of catalase (CAT), ascobate peroxidase (APX), peroxidase (POD), and superoxide dismutase (SOD) activities in broccoli florets stored at 15 °C. Florets were treated with distilled water (control) and 200 mg/L 6-BA for 1 min at 20 °C. Each data point is the mean of three replicate samples. Vertical bars represent standard errors of the means.

throughout the whole storage period. On the other hand, the APX activity in florets of the 6-BA-treated broccoli increased on the first day and then decreased (Figure 4B). When compared to the control florets, 6-BA treatment maintained notably ($P \leq 0.05$) higher activity of APX during storage. POD activity increased in the control broccoli florets during the first 2 days of storage, fell during the following day, and then increased sharply on the fourth day. In contrast, the activity in 6-BA-treated broccoli exhibited a slight decrease during the first 3 days and increased rapidly thereafter, but to a lower extent than in the control (Figure 4C). SOD activity declined gradually in all broccoli florets regardless of treatment (Figure 4D). 6-BA treatment significantly improved the SOD activity, which made the activity always higher in the treatment than in the control throughout the whole storage period.

Effect of 6-BA Treatment on DPPH Radical-Scavenging Activity and Total Phenols in Broccoli Florets. The DPPH radical-scavenging activity increased in 6-BA-treated samples during storage, whereas the activity in control samples decreased at the first day, then increased gradually in the next 2 days, and thereafter decreased again (Figure 5A). The florets treated with 6-BA showed relatively higher DPPH radical scavenging activity in comparison with the control florets throughout the whole storage period.

The content of phenolic compounds increased constantly in 6-BA-treated broccoli florets until the end of the storage period, whereas the phenolic content in control florets decreased at the first day and increased thereafter (Figure 5B). The phenolic content in the 6-BA-treated broccoli was significantly ($P \leq 0.05$) higher than that in the control during the whole storage.

Effect of 6-BA Treatment on Total Glucosinolate and Sulforaphane Contents in Broccoli Florets. The content of total glucosinolate in both 6-BA-treated and control broccoli increased on the first day and then decreased gradually during storage (Figure 6A). 6-BA treatment maintained a profoundly ($P \leq 0.05$) higher total glucosinolate content across the whole storage period than did the control.

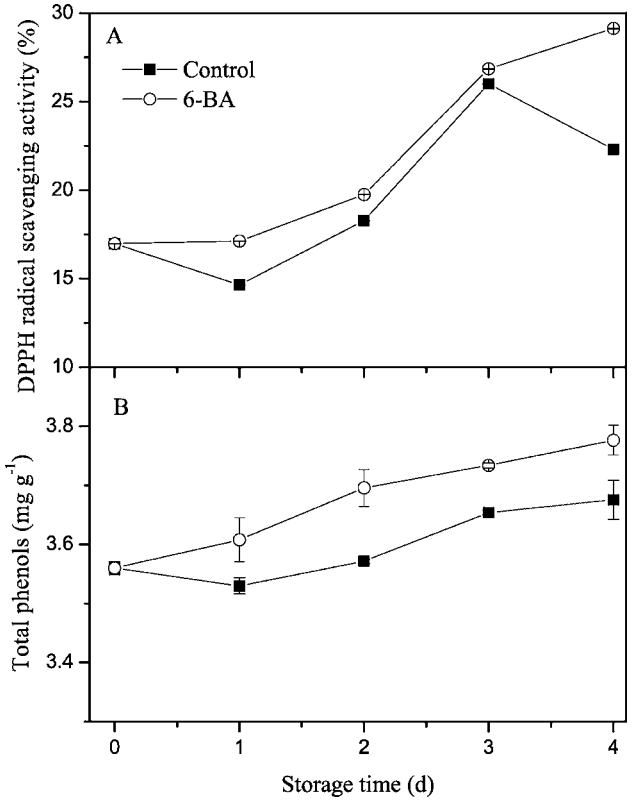

Figure 5. Changes of DPPH radical scavenging activity (A) and total phenol content (B) in broccoli florets stored at 15 °C. Florets were treated with distilled water (control) and 200 mg/L 6-BA for 1 min at 20 °C. Each data point is the mean of three replicate samples. Vertical bars represent standard errors of the means.

The content of sulforaphane in the control broccoli florets tended to decrease gradually until 3 days in storage and then increased slightly (Figure 6B). On the other hand, the sulforaphane content in 6-BA-treated broccoli increased on the first day and then declined gradually until the end of the

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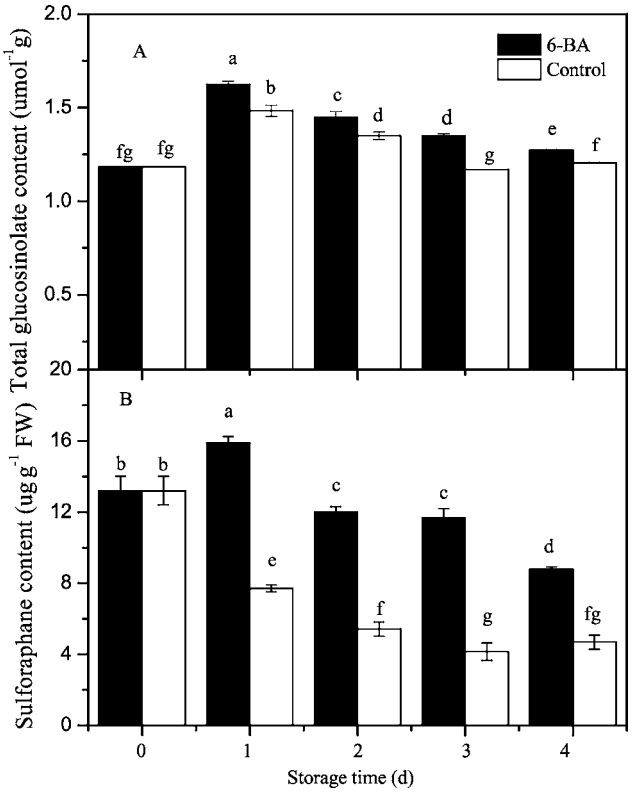

Figure 6. Changes of total glucosinolate (A) and sulforaphane contents (B) in broccoli florets stored at 15 °C. Florets were treated with distilled water (control) and 200 mg/L 6-BA for 1 min at 20 °C. Each data point is the mean of three replicate samples. Vertical bars represent standard errors of the means.

storage period. Throughout the whole storage period, treatment with 6-BA maintained a higher level of sulforaphane in florets in comparison with the control.

DISCUSSION

During the postharvest period, the most obvious characteristic of senescence in broccoli is floret yellowing.[27] Our work showed that 6-BA treatment significantly inhibited the increase of L^* value and caused the higher retention of H value as compared with the control florets. This is consistent with the findings of Down et al.[14] and Tian et al.[15]

The activity of chlorophyllase has been proposed to be the first step in chlorophyll degradation, and the second step would be the elimination of Mg^{2+} from chlorophyllase to produce pheophorbide, in a reaction catalyzed by Mg-dechelatase.[28] Heat treatment reduced the chlorophyll degradation through the suppression of activities of chlorophyll catabolic enzymes, including chlorophyllase, Mg-dechelatase, chlorophyll-degrading peroxidase, and chlorophyll oxidase.[17,29] Costa et al.[30] found that the increment of chlorophyllase activity during broccoli senescence is up-regulated by ethylene and down-regulated by cytokinins. Our study also supported the evidence that treatment of broccoli florets with 6-BA inhibited chlorophyll degradation and reduced chlorophyllase and Mg-dechelatase levels.

MDA is the product of membrane peroxidation and could damage the structure and integrity of membrane during the senescence of broccoli florets. Postharvest senescence of broccoli is correlated with lipid peroxidation (MDA content), leading to cell-membrane disintegration.[31] Yuan et al.[5] reported that 1-MCP treatment could inhibit the increase of MDA amount in broccoli florets during postharvest storage. It was found in our work that 6-BA treatment delayed the increase of MDA content compared to control florets. It is possible that 6-BA could prohibit loss of membrane integrity and mixing of glucosinolates with myrosinase, leading to less glucosinolate hydrolysis.

The mechanisms to prevent oxidation are associated with the defense system, including antioxidant enzymes and endogenous antioxidation, both of which are believed to be important in preventing oxidative injury through their abilities to scavenge free radicals before they cause cellular damage. The coordinated action of antioxidant enzymes such as SOD, APX, and CAT helps to reduce oxidative damage in senescence and regeneration of ascorbate and glutathione metabolites.[32] SOD catalyzes the dismutation of superoxide radical to O_2 and H_2O_2; CAT and APX then catabolize H_2O_2 to H_2O and O_2 and therefore limit the potential for further free radical production from H_2O_2.[33] In our present study, when compared with the control florets, the activities of CAT, SOD, and APX were higher in florets treated with 6-BA during storage time (Figure 4). Toivonen et al.[20] reported that protection offered by antioxidant enzymes is important for the retention of green color in broccoli flower buds, and the increases in antioxidant enzyme activities were likely defense responses to the increases in oxygen radical production, which could subsequently lead to yellowing of broccoli. Peroxidase mediates chlorophyll degradation in the presence of a phenolic compound, through the following possible mechanism: the enzyme catalyzes the oxidation of a phenolic compound with hydrogen peroxide, generating a phenolic radical, which in turn degrades chlorophyll to a colorless compound.[34,35] Moreover, Funamoto et al.[17] found that the delay in chlorophyll degradation was closely related to a lower peroxidase activity in heat-treated samples. According to Costa et al.,[30] highly active peroxidase and hydrogen peroxide could mediate degradation of chlorophylls. In this study, we found that POD activity in 6-BA-treated broccoli florets was lower than in the control (Figure 4C). These results implied that reduction of oxidative stress in broccoli florets might be one reason 6-BA treatment was effective in delaying senescence.

The level of antioxidants plays a critical role in human health maintenance by preventing oxidative damage to molecules and cells. 6-BA treatment can increase the antioxidant capacity, whereas the DPPH radical-scavenging activity decreased in control broccoli florets (Figure 5A). It is probable that the storage of control florets for 15 °C for 4 days induced a more intense senescence than in 6-BA-treated florets, leading to a higher reduction in antioxidant capacity. In the present study, higher levels of total phenols were detected in 6-BA-treated samples. One of the key enzymes in phenolic synthesis is phenylalanine ammonia-lyase (PAL), the activity of which could be directly related to the content of phenolic compounds.[36] Previous works have shown that heat treatments can reduce PAL activity in strawberry[37] and that induced by sucrose in broccoli sprouts.[10]

Glucosinolates, one of the main health-promoting secondary metabolites in *Brassica*, are a group of sulfur- and nitrogen-containing secondary metabolites.[5] Cutting of broccoli heads to broccoli florets brings myrosinase in contact with glucosinolates, which might lead to a high degree of glucosinolate hydrolysis. Jia et al.[38] and Yuan et al.[5] speculated that the glucosinolate level of stored broccoli florets is a reflection of

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two opposing mechanisms: hydrolysis of glucosinolate by myrosinase and induction of glucosinolate biosynthesis by an unknown mechanism. Cytokinin regulated postharvest senescence in broccoli through nullifying the perception of ethylene and reducing the effect of ethylene on the up-regulation of senescence-associated gene expression.[39] The present study showed that 6-BA treatment leads to better retention of total glucosinolate content, in comparison with the control during storage (Figure 6A). It is possible that the blockage of ethylene action by 6-BA treatment favored the biosynthesis of glucosinolates or inhibited some ethylene-related degradative pathways. It is necessary to explore how 6-BA affects the glucosinolate biosynthesis pathway at the molecular level.

Sulforaphane, an isothiocyanate from broccoli, is one of the most potent food-derived anticarcinogens. A common assumption in the current literature regarding the health benefits of sulforaphane from broccoli is that glucoraphanin is converted to sulforaphane when broccoli is crushed.[11] However, a number of studies have demonstrated that the sulforaphane yield from glucoraphanin is low and that a nonbioactive nitrile analogue, sulforaphane nitrile, is the primary hydrolysis product when plant tissue is crushed at room temperature. However, sulforaphane nitrile has recently been shown not to possess the anticarcinogenic properties of sulforaphane.[40] Thus, it should be noted that the optimum hydrolyzed condition is how much glucoraphanin may be converted to bioactive sulforaphane rather than sulforaphane nitrile. It has been reported that the formation of isothiocyanates in broccoli can be promoted by high pressure[41] and that the sulforaphane content in broccoli was also affected by different cooking methods.[42] Sucrose and mannitol treatments have also been shown to increase the content of sulforaphane in broccoli sprouts.[10] In our current study, the sulforaphane content in broccoli florets treated with 6-BA was significantly increased (Figure 6B). In this regard, it is likely that 6-BA treatment can improve the substrate (glucoraphanin) in broccoli florets. In the study by Chiang et al.,[43] in which sulforaphane production predominated over the nitrile, some samples were mixed with exogenous myrosinase prior to hydrolysis, therefore, it is possible that exogenous myrosinase added during hydrolysis may have favored sulforaphane production. Further studies are needed to explore the mechanism of sulforaphane formation in broccoli florets treated by 6-BA.

In conclusion, this study showed that 6-BA treatment not only maintained visual quality and retarded the chlorophyll degradation in broccoli florets but also improved the antioxidant capacity and the antioxidant enzyme activities including CAT, SOD, and APX. The contents of total phenolic compounds, glucosinolate, and sulforaphane in broccoli florets were significantly enhanced by 6-BA treatment. These results implied that treatment with 6-BA could produce more health-promoting compounds and enhance the nutritional value of broccoli. Further studies are warranted to investigate the specific mechanisms of how 6-BA treatment maintains the elevated levels of glucosinolate and sulforaphane and delays the senescence of broccoli florets.

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