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Exogenous sodium nitroprusside treatment of broccoli florets extends shelf life, enhances antioxidant enzyme activity, and inhibits chlorophyll-degradation

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ABSTRACT

The effect of an exogenous application of sodium nitroprusside (SNP) on the shelf life, antioxidant enzyme and chlorophyll-degrading enzyme activity, and chlorophyll-degradation related gene expression was investigated in stored broccoli. The preliminary results indicated that the 200 μmol L^{-1} SNP treatment had the greatest effect on extending shelf life of broccoli florets so this concentration was used in the remainder of the study. The SNP treatment delayed chlorophyll degradation, thus color was maintained and shelf-life was extended. The activity of the antioxidant enzymes, catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) was all enhanced while glutathione reductase (GR) activity was inhibited throughout the storage. Relative to the untreated control, the SNP treatment suppressed the activity of the chlorophyll-degrading enzymes, chlorophyllase (Chlase), chlorophyll-degrading peroxidase (Chl-POX), Mg-dechelatase (MD), and pheophytinase (PPH), and also suppressed chlorophyllase I (*BoCHL1*), chlorophyllase II (*BoCHL2*), chlorophyllase III (*BoCLH3*) and pheophorbide a oxygenase (*BoPAO*) gene expression during the entire storage period. In summary, 200 μmol L^{-1} SNP treatment of broccoli extends shelf life, enhances oxidative stress tolerance by enhancing the activity of antioxidant enzymes, and inhibits the activity of chlorophyll-degrading enzymes and related gene expression. The combined effect delayed the yellowing of broccoli florets by inhibiting chlorophyll degradation, thus extending the shelf life of broccoli florets.

1. Introduction

Broccoli (*Brassica oleracea* var. Italica), a cruciferous vegetable, contains a high level of antioxidant compounds. It is also rich in vitamins, and anti-carcinogenic compounds, such as glucosinolates, and sulforaphane nitrile (Williams et al., 2008). However, it is a highly perishable product, and florets undergo rapid yellowing at room temperature after harvest (Hansen et al., 2001), thus reducing the shelf life of the florets and resulting in a low-quality product (Funamoto et al., 2006). The yellowing of broccoli florets, the symptom of postharvest senescence, is accompanied by a loss in fresh weight, chlorophyll content, sugars, and proteins (Costa et al., 2005). There is also a loss in aroma, a decrease in amino acid content (Hansen et al., 2001), a decrease in total aliphatic and indole glucosinolates, and a general reduction in antioxidant compounds (Jia et al., 2009). Additionally, lipid peroxidation and protein degradation increased (Page et al., 2001). Oxidative processes play an important role in plant senescence, thus antioxidant enzymes, such as catalases (CAT), superoxide dismutases (SOD), peroxidases (POD), and the ascorbate-glutathione, have evolved in plants to combat oxidative stress and delay senescence (Lemoine et al., 2010).

Yellowing of broccoli florets results from a loss and degradation of chlorophyll (Chl). Chl a is degraded by enzymes that include chlorophyllase (Chlase) (Harpaz-Saad et al., 2007), chlorophyll-degrading peroxidase (Yamauchi et al., 2004), Mg-dechelatase (MD) (Suzuki et al., 2005), or another Mg-dechelating substance (MDS), pheophorbide (pheide) a oxygenase (PaO), and red Chl

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catabolite reductase (Hörtensteiner, 2006). Chl degradation occurs mainly within the chloroplast. Moreover, the peroxidase-mediated Chl degradation may also occur in the vacuole (Yamauchi et al., 2004; Hörtensteiner, 2006). Several approaches have been used to delay the senescence of broccoli florets, including heat treatment (Lemoine et al., 2010), modified atmosphere packaging (Jia et al., 2009), UV irradiation (Costa et al., 2006; Aiamla-or et al., 2009, 2012), and chemical treatments such as ozone and 1-methylcyclopropene (1-MCP) (Forney et al., 2003).

Nitric oxide (NO) is an important signaling molecule with diverse physiological functions for plants, participating in various cellular processes, such as respiratory metabolism, growth and development, as well as maturation and senescence (Lamattina et al., 2003). Some researchers found that NO gas or sodium nitroprusside (SNP, as a donor of exogenous NO) treatment, delayed ripening, senescence and enhanced the resistance in horticulture crops (Wills et al., 2008; Wu et al., 2014; Iakimova and Woltering, 2015). For instance, Leshem et al. (1998) originally reported that NO-treated broccoli markedly retained green color and firmness compared to the air control and the ethylene-treated broccoli. The senescence-delaying effects of NO were also observed in carnation, Geraldton wax flowers (*Chamelaucium uncinatum* Schauer) and waratah flowers (*Telopea speciosissima*, Proteaceae) (Zeng et al., 2011; Leshem et al., 1998). However, the metabolic and physiological responses of broccoli to NO treatment remains still poorly understood.

The overall objective of present study was to determine the effect of NO treatment (via the exogenous application of SNP) on broccoli florets stored at 20 °C. More specifically, the effects of SNP on broccoli floret quality parameters, antioxidant enzyme and chlorophyll-degrading enzyme activity, as well as chlorophyll-degradation related gene expressions during storage were investigated.

2. Materials and methods

2.1. Plant material and treatments

Broccoli (*B. oleracea* var. Italica, 'Bao-shi') was harvested from an organic vegetable farm in the town of Xiaotangshan, located in the Shunyi District of Beijing, China, top-iced and directly brought to the laboratory within 3 h. The selected broccoli ranged in diameter from 15 to 20 cm, were uniform in color, had no evidence of insect or disease injury, no mechanically-induced wounds, and all had tight-head clusters.

The selected broccoli heads were randomly placed into five groups. One group was treated with distilled water (control) and four groups received exogenous application of 100, 200, 400, and 800 $\mu mol \cdot L^{-1}$ sodium nitroprusside (SNP). Broccoli heads were air dried and then placed in polyethylene film bags (0.03 mm in thickness), with the top folded over, placed in plastic crates, and stored in the dark at 20 ± 1 °C and 90% RH. An assessment of shelf life, and a visual color scale rating were made daily in replicates of nine broccoli heads per treatment. The florets, together with about 1 cm of stalk, were cut from the broccoli heads each day and served as a sampling unit. The samples were immediately frozen in liquid nitrogen and stored at −80 °C. The frozen florets were used to determine chlorophyll content, antioxidant enzyme activity, chlorophyll-degrading enzyme activity, and the relative expression of genes coding for proteins involved in chlorophyll degradation.

2.2. Determination of shelf life

The shelf life of broccoli florets was determined according to the previous study (Yuan et al., 2010). A percentage of ≥30% yellowing florets was designated as the end of shelf life.

2.3. Measurement of visual color rating scale

The color of broccoli florets was measured by a visual assessment of changes from green to yellow as previously described (Rangkadilok et al., 2002). A color rating scale from 0 to 9 was adopted to indicate the general percentage of yellowing in the broccoli head, where 0 indicates all dark green, 1 indicates 3–5% yellowing, 5 indicates 50% yellowing of the head, 7 indicates 75% yellowing of the head, and 9 indicates 100% yellowing of the broccoli heads.

2.4. Total chlorophyll content

Frozen floret tissues were homogenized in acetone:ethanol (2:1) using a mortar and pestle. The homogenate was then filtrated and the filtrate was used to measure chlorophyll content according to Sun et al. (2012). The optical density at 645 nm and 643 mm were recorded for each sample.

2.5. Antioxidant enzyme activity

Three grams of frozen flower tissue was extracted with 15 mL 0.1 $mol \cdot L^{-1}$ phosphate buffer (PBS, pH 7.8, containing 0.5% polyvinylpyrrolidone), then centrifuged at 12,000 × *g* for 20 min at 4 °C. The supernatant was collected and used for enzyme assay.

Catalase (CAT) activity was measured by determining the decrease in absorbance at 240 nm due to H_2O_2 consumption as described by Azevedo et al. (2007). The reaction mixture contained: 1.0 mL of 0.3% H_2O_2, 1.9 mL of 0.05 $mol \cdot L^{-1}$ phosphate buffer (PBS, pH 7.8), and 0.1 mL of sample.

Peroxidase (POD) activity was measured according to Bai et al. (2006). One gram of frozen floret tissue was homogenized in 8.0 mL of 0.05 M PBS (pH 7.8). The reaction mixture contained: 1.0 mL of 0.3% H_2O_2, 1.0 mL of 0.05 M PBS (pH 7.8), 0.9 mL of 0.2% guaiacol, and 0.1 mL of sample. The increase in absorbance at 470 nm over one minute was determined.

Glutathione reductase (GR) activity was assayed by measuring NADPH oxidation in the presence of oxidized glutathione (Ognjanović et al., 2010). The reaction mixture consisted of 2.7 mL PBS (pH 7.5, containing 1 mmol L^{-1} EDTA), 0.1 mL of 5 mmol L^{-1} oxidized glutathione, 0.2 mL of sample, and 40 μL of 4.0 mmol L^{-1} NADPH. GR activity was determined as a change in absorbance at 340 nm over 1 min.

Ascorbate peroxidase (APX) activity was determined by measuring the decrease in absorbance of ascorbic acid (AsA) at 290 nm (Zhang et al., 2010). The reaction mixture contained 2.6 mL of PBS (pH 7.5, containing 0.1 mmol L^{-1} EDTA and 0.5 mmol L^{-1} AsA), 0.1 mL of sample, and 0.3 mL of 2 mmol L^{-1} H_2O_2.

2.6. Chlorophyll-degrading enzyme activity

An acetone-derived powder of floral tissues was prepared by the method of Aiamla-or et al. (2012). About 1 g broccoli floral tissues was homogenized in cold acetone (−20 °C). The homogenate was placed at −20 °C for 15 min, then centrifuged at 12,000 × *g* for 5 min at 4 °C. The precipitate was then washed twice with cold acetone. The resulting precipitate was used as the source of acetone powder in the following assays. Chl a was bought from sigma company, and then 200 μg mL^{-1} Chl a was prepared in acetone. Chlorophyllin a (Chlin) was prepared with 30% KOH in methanol from chl a in petroleum ether. Pheophytin (Phy) a was prepared by the acidic reaction from Chl a, using 0.1 M hydrochloric acid. The Phy a concentration was measured spectrophotometrically at 409 nm (Aiamla-or et al., 2012).

Chlorophyll-degrading enzymes were extracted and measured as described by Aiamla-or et al. (2010).