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Reducing yellowing and enhancing antioxidant capacity of broccoli in storage by sucrose treatment

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ABSTRACT

The effect of sucrose treatment on shelf life, the color, contents of glucosinolate, sulforaphane, total phenols, and antioxidant activity in broccoli florets was investigated. The results showed that sucrose ($12\,g\,L^{-1}$) treatment extended shelf life, inhibited the increase of L^* value, retained a high hue angle and chlorophyll content. The decreases of glucosinolate and sulforaphane contents in broccoli florets were suppressed after sucrose treatment. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity and the activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), phenylalanine ammonia-lyase (PAL) and catalase (CAT) were enhanced in sucrose-treated florets, whereas the activity of peroxidase (POD) was significantly reduced. Sucrose treatment also enhanced total phenolic content and maintained higher levels of total and reducing sugars. These results indicated that sucrose treatment can prolong shelf life in non-refrigerated retail display cases, maintain quality and improve the nutritional value of postharvest broccoli florets.

1. Introduction

Broccoli (*Brassica oleracea* var. *italica*) contains considerable amount of antioxidants, vitamins, and it has long been consumed globally as an anti-cancer vegetable, which is a rich source of glucosinolates, especially sulforaphane (Volden et al., 2009; Qin et al., 2009). Sulforaphane is formed from its glucosinolate (glucoraphanin) by myrosinase when broccoli tissue is crushed or chewed. More epidemiological studies have shown that sulforaphane has antitumor activity by enhancing detoxification of carcinogens and blocking the initiation of chemically induced carcinogenesis in animal models (Gills et al., 2006; Zhang, 2004).

Broccoli is harvested when the flowering heads are immature (Tian et al., 1994). Broccoli florets senescence and turn yellow rapidly at room temperature after harvest, resulting in the losing of their commercial value (Jia et al., 2009). Thus, many storage techniques have been widely explored to extend shelf life and improve quality in postharvest broccoli, such as the application of ethanol, 1-methylcyclopropene (1-MCP), and 6-benzylaminopurine (6-BA) treatment (Xu et al., 2012a,b; Yuan et al., 2010).

Sugars as sources of carbon skeleton are necessary to maintain energy supply and extend the postharvest life of perishable horticultural commodities. It has been shown that exogenous sucrose supply can delay postharvest senescence and increase the shelf life of broccoli (Irving and Joyce, 1995). In leaves of roquette, the extension in postharvest shelf life was related to higher sucrose level (Clarkson et al., 2005). Guo et al. (2011b) reported that sucrose may act as a key signaling molecule in inducing the accumulation of glucosinolates and anthocyanins in broccoli sprouts. It was further reported that exogenous sucrose treatment in broccoli can improve postharvest quality through altering ethylene metabolism (Nishikawa et al., 2005), maintaining higher level of chlorophyll in the florets (Coupe et al., 2003), and enhancing ascorbic acid contents (Smirnoff and Pallanca, 1996). Nonetheless, the information about exogenous sucrose treatment on antioxidant capacity and active compounds of postharvest broccoli florets is limited. Our aim of the present study was to investigate the effect of sucrose treatment on visual quality, antioxidant enzyme activities and health-promoting compounds (glucosinolates and sulforaphane) in broccoli florets during storage at 20 °C.

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2. Materials and methods

2.1. Plant materials and treatment

Fresh broccoli (*Brassica oleracea* L. var. *Italica*, cv. Youxiu) heads were harvested in Ningbo, Zhejiang Province, China. Broccoli heads were placed on ice and carried back to the laboratory within 5 h. They were picked for uniform size, color and absence of defects for two experiments. In experiment I, broccoli heads with stem were randomly divided into 8 groups 4 heads in each group. Each group of heads was respectively placed in plastic boxes for continuous sucrose treatment all the time. Sucrose solution in the container was sufficient so that the bottom of the stem could be submerged below the liquid surface. Eight different sucrose concentrations (0, 4, 6, 8, 10, 12, 14, 16 g L^{-1}, containing a volume fraction of 0.05% sodium hypochlorite) were utilized. The broccoli heads were covered with polyethylene film to reduce water loss and stored at 20 °C at 95% relative humidity and solutions were renewed daily. Shelf life was determined. It was found that florets treated with 12 g L^{-1} sucrose had the longest shelf life. Therefore, this concentration was used in experiment II, three replicates of 30 heads per treatment were used, and the whole experiment was repeated twice. Samples were taken before treatment (time 0) and daily during storage, and florets were removed from the stems. Then the florets were immediately frozen in liquid nitrogen and kept at −20 °C for later analysis.

2.2. Shelf life and color evaluation

Shelf life was analyzed according to the method by Ku and Wills (1999). The time for quality to decline to 30% yellowing in florets was determined as the limit for their shelf life. Five points over the surface of a floret were randomly selected for measuring the color of broccoli florets with a colorimeter (Minolta CR-400, Osaka, Japan). The hue angle (H) was calculated as $h^0 = \tan^{-1}(b/a)$ when $a > 0$ and $b > 0$ or $h^0 = 180^0 + \tan^{-1}(b/a)$ when $a < 0$ and $b > 0$. The lightness value (L^*) indicates how dark/light the sample is (varying from 0, black, to 100, white).

2.3. Chlorophyll content

Chlorophyll content was determined as previously described with minor modifications (Yuan et al., 2010). Chlorophyll quantification was measured at 665 and 649 nm and the result was expressed as milligrams of chlorophyll mass per gram of fresh weight.

2.4. Determination of sulforaphane and total glucosinolate content

Sulforaphane was extracted from broccoli samples according to the method of Liang et al. (2006). Sample (0.2 g) was milled with liquid nitrogen in a precooled mortar and mixed with 2 mL 0.5 U mL^{-1} myrosinase solution. Acetonitrile (1 mL) was added to the liquid for dissolving the residue. And then the liquid was passed through a 0.45 μm membrane filter before injecting into HPLC. Samples were separated at 30 °C on a Kromasil C18 column (250 × 4.6 mm) using acetonitrile and water at a flow rate of 1.0 mL min^{-1}. Sulforaphane (Sigma) was used as an external standard for HPLC analysis. Absorbance was measured at 254 nm.

Total glucosinolate content was determined according to the method of Heaney et al. (1988). The amount of glucose was measured by the method of phenol-sulphuric acid with analyzing the absorbance at 490 nm. The glucosinolate content was calculated from the glucose content.

2.5. Antioxidant enzyme assays

The antioxidant enzyme activities were determined according to the method of Toivonen and Sweeney (1998) with slight modifications. Frozen broccoli floret (0.2 g) was milled with 5 mL of (50 mM, pH 7.8) phosphate buffer in a precooled mortar. The resultant homogenate was centrifuged at 12,000 × g for 15 min at 4 °C. The supernatant was held for the further antioxidant enzyme activities measurement.

CAT activity was determined by monitoring the enzyme-catalyzed decomposition of H_2O_2 by potassium permanganate. One unit of CAT activity was defined as the amount of enzyme that decomposes l mol H_2O_2 min^{-1} at 30 °C. SOD activity was measured based on the photoreduction of tetranitroblue tetrazolium chloride (NBT) by light in the presence of riboflavin and methionine. One unit of SOD was the amount of extract that gave 50% inhibition of the rate of reduction NBT. Ascorbate peroxidase (APX) activity measurement was adapted from Vicente et al. (2006). One unit of APX enzyme activity was defined as the amount of enzyme that produced an OD_{290} reduction per min under the assay conditions. POD activity was measured based on the determination of guaiacol oxidation at 470 nm by H_2O_2. The change in absorbance at 470 nm was followed every 30 s by spectrophotometer. PAL activity was determined according to the method of Kim et al. (2006). The antioxidant enzyme activities were expressed as units per milligram protein. Protein content was measured according to the method of Bradford (1976).

2.6. Determination of DPPH radical-scavenging activity and total phenolic content

DPPH radical-scavenging activity was measured according to the method of Hatano et al. (1988). The absorbance was measured at 550 nm, result was calculated according to the formula: DPPH radical scavenging activity (%) = 100 − (absorbance of sample/absorbance of control) × 100. Total phenolic content was determined according to the Folin–Ciocalteu procedure described by Slinkard and Singleton (1977). The absorbance was measured at 765 nm. Results were expressed as milligram of gallic acid equivalent per gram fresh weight.

2.7. Determination of total soluble sugar and reducing sugar contents

Frozen broccoli florets (5 g) were extracted in 5 mL distilled water and incubated at 85 °C for 30 min, then centrifuged at 10,000 × g at 4 °C. Reducing sugar content was assayed spectrophotometrically at 540 nm with 3,5-dinitrosalicylic acid by Wang (2005). Total soluble sugar content was determined using anthrone reagent and glucose as standard.

2.8. Statistical analysis

Statistical analysis was performed using the SPSS package program version 19.0 (SPSS Inc., Chicago, IL, USA). Data was analyzed by one-way ANOVA. The values are reported as means with their standard error for all results. Main effects were analyzed and means were compared by Duncan's multiple range tests at a significance level of 0.05.

3. Results

3.1. Effect of different concentrations of sucrose solution on shelf life in broccoli florets

As shown in Fig. 1, the shelf life of broccoli florets increased with increasing sucrose solution concentration. However, high