Food Chemistry 217 (2017) 45–51

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effects of 24-epibrassinolide on enzymatic browning and antioxidant activity of fresh-cut lotus root slices

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ARTICLE INFO

Article history:
Received 26 March 2016
Received in revised form 27 July 2016
Accepted 22 August 2016
Available online 24 August 2016

Keywords:
Fresh-cut lotus root slices
Browning
Brassinosteroids
Phenolic metabolism
Enzymatic and non-enzymatic antioxidants

ABSTRACT

Fresh-cut lotus root slices were treated with 80 nM 24-epibrassinolide (EBR) and then stored at 4 °C for 8 days to investigate the effects on cut surface browning. The results showed that EBR treatment reduced cut surface browning in lotus root slices and alleviated membrane lipid peroxidation as reflected by low malondialdehyde content and lipoxygenase activity. EBR treatment inhibited the activity of phenylalanine ammonia lyase and polyphenol oxidase, and subsequently decreased phenolics accumulation and soluble quniones formation. The treatment also stimulated the activity of peroxidase, catalase and ascorbate peroxidase and delayed the loss of ascorbic acid, which would help prevent membrane lipid peroxidation, as a consequence, reducing decompartmentation of enzymes and substrates causing enzymatic browning. These results indicate that EBR treatment is a promising attempt to control browning at cut surface of fresh-cut lotus root slices.

1. Introduction

Lotus root, the edible rhizome of *Nelumbo nucifera*, is a very popular aquatic vegetable consumed worldwide being the rhizome is crisp in texture and has an attractive aroma, white color and enriched with an excellent source of nutritional components. In recent years, consumption/production of fresh-cut lotus root has continuously increased as more consumers demand convenient and ready-to-eat foods (Sun et al., 2015; Zhang, Yu, Xiao, Wang, & Tian, 2013). However, the quality and consumer acceptance of fresh-cut lotus root is often limited by cut surface browning during shelf-life. Enzymatic oxidation of phenolics, membrane lipid peroxidation and reactive oxygen species (ROS) overproduction or scavenger system failure have been demonstrated to be responsible for browning of lotus root slices (Sun et al., 2015; Zhang et al., 2013). Therefore, pre-attempts that are beneficial to inhibit these physiological processes could be effective in browning inhibition. As expected, chlorine dioxide (Du, Fu, & Wang, 2009), carbon monoxide (Zhang et al., 2013), hydrogen sulfide (Sun et al., 2015) and combined application of chitosan based coating and modified atmosphere packaging (Xing, Li, Jiang, Yun, & Li, 2010) and synergistic effect of ascorbic acid, heat treatment and modified atmosphere packaging (Son, Hyun, Lee, Lee, & Moon, 2015) have been examined with satisfactory results.

Brassinosteroids (BRs) are essential plant hormones that have crucial roles in plant growth and development, as well as in plant responses to abiotic and biotic stresses (Bajguz & Hayat, 2009). It is clear that modulating BRs level or sensitivity can improve crop plants productivity and stress adaptation (Xia et al., 2009). More recent work, however, provides evidence that BRs exposure is a promising and efficient attempt for postharvest horticultural products to preserve storage quality. For example, after jujube fruit were immersed postharvest in 5 μM brassinolide for 5 min, the development of blue mold was inhibited effectively (Zhu, Zhang, Qin, & Tian, 2010). Zhu et al. (2015) arrived at a similar conclusion that the disease incidence of Satsuma mandarin fruit can be significantly reduced by 24-epibrassinolide (EBR). Additionally, BRs treatment has been suggested to enhance chilling tolerance in harvested tomato (Aghdam, Asghari, Farmani, Mohayeji, & Moradbeygi, 2012), mango (Li et al., 2012) and green bell pepper (Wang, Ding, Gao, Pang, & Yang, 2012) fruits. Also, in previous studies we have suggested that EBR ameliorated chilling-induced physiological disorders, especially pulp browning, the typical chilling injury symptom in eggplant (Gao et al., 2015) and peach (Gao et al., 2016) fruits. Another very recent work by Liu et al. (2016) showed the benefit of BRs exposure in preventing flesh browning in bamboo shoot as well. It seems worthwhile, therefore, to investigate BRs for regulating cut surface browning in fresh-cut lotus root slices. However, there are no reports on the effects of BRs on lotus root slices.

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http://dx.doi.org/10.1016/j.foodchem.2016.08.063
0308-8146/

The objective of this study was to investigate the effects of EBR treatment on enzymatic browning of fresh-cut lotus root slices during storage. The study specifically addressed the effects of EBR on changes in color and overall visual quality and physiological parameters including membrane effects and prooxidant and antioxidant systems. The results can help to better understand the role of BRs in anti-browning and provide a new attempt for lotus root slices to maintain quality and extend shelf life.

2. Materials and methods

2.1. Plant material and treatments

Lotus roots (*N. nucifera* Gaertn. cv. Elian V) were obtained from a local wholesale market at Xi'an, China; all were of uniform size and ground color and without mechanical damage. After pre-cool at 4 °C for 24 h, the lotus roots were washed, peeled and sliced into 4 mm thick disks. Then the lotus root slices were divided randomly into 2 treatment groups, with 800 g for each treatment. The two groups of lotus root slices were immersed in distilled water (control) and 80 nM aqueous solution of EBR at room temperature for 2 min. This EBR concentration was determined to be the optimum based on preliminary experiments using 0, 20, 40, 60, 80 and 100 nM EBR. All the treated lotus root slices were held over a plastic sieve for 10 min to drain excess solution. After this, they were placed into polyethylene bags and stored at 4 °C and 85–90% relative humidity which was measured using a digital humidometer. For each treatment, three replicates were used. Samples were taken after slicing (day 0) and at four successive 2 day intervals during storage for subsequent assessments.

2.2. Measurement of color and overall visual quality

The surface color change of slices was determined using a chroma meter (SC-80C, Kangguang Instrument Co., Ltd., Beijing, China), which presented the L^*, a^* and b^* values by the CIE color system.

The overall visual quality was evaluated according to Du et al. (2009). The sensory evaluation standard was as follows: 9 (excellent, with no defects), 7 (good, with minor defects), 5 (fair, with moderate defects), 3 (poor, with major defects) and 1 (unusable). A score of 6 was considered to be the limit of salability and the shelf-life was defined.

2.3. Measurement of malondialdehyde (MDA) content

MDA content was measured by a modified method of Dhindsa, Pulmb-Dhindsa, and Thorpe (1981). Lotus root tissue (2 g) was homogenized with 10 mL of 10% trichloroacetic acid containing 0.5% (w/v) thiobarbituric acid. The mixture was heated at 100 °C for 10 min, cooled quickly by running water, and then centrifuged at 5000×*g* for 15 min. Absorbance of the supernatant was measured at 450, 532 and 600 nm. MDA content was expressed on a fresh weight basis as μmol g^{-1}.

2.4. Measurement of total phenolic and soluble quinone contents

Lotus root tissue (5 g) was homogenized in 20 mL of pure methanol. After centrifugation, the supernatant was collected and used as crude extract. For total phenolic content determination, the diluted crude extract of 0.2 mL was gently mixed with 1.0 mL of Folin–Ciocalteu reagent and 3 mL of sodium carbonate (1 M) and the total volume of the mixture were adjusted to 10 mL with distilled water. After the mixture had been kept at room temperature for 1 h, the absorbance was read at 760 nm (Hinneburg, Dorman, & Hiltunen, 2006). Results were expressed as the mass of gallic acid equivalents on a fresh weight basis in mg g^{-1}.

For soluble quinone content determination, the crude extract was used directly and recorded absorbance at 437 nm (Banerjee, Penna, & Variyar, 2015). Results were expressed on a fresh weight basis as OD437 g^{-1}.

2.5. Measurement of ascorbic acid content

Lotus root tissue (5 g) was homogenized in 20 mL of 2% oxalic acid solution and centrifuged at 8000×*g* and 4 °C for 15 min. Ten mL supernatant was titrated with a calibrated 2,6-dichlorpphenol indophenols solution until a permanent pink color (Bessey & King, 1933). Results were expressed on a fresh weight basis as mg g^{-1}.

2.6. Measurement of enzyme activity

Lotus root tissue (2 g) was homogenized in various precooled buffers (4 °C) to prepare crude extracts for assay of the following enzymes: 8 mL of 50 mM sodium phosphate buffer (pH 6.8) containing polyvinylpyrrolidone for polyphenol oxidase (PPO), peroxidase (POD), catalase (CAT) and lipoxygenase (LOX); 5 mL of 0.2 M borate buffer (pH 8.8) containing of polyvinylpyrrolidone for phenylalanine ammonia lyase (PAL); 8 mL of 100 mM potassium phosphate buffer (pH 7.5) containing 0.1 mM ethylene diamine tetraacetic acid, 1 mM ascorbic acid and polyvinylpyrrolidone for ascorbate peroxidase (APX). The tissue homogenates were then centrifuged at 12,000 × *g* and 4 °C for 15 min. The supernatants were used for the enzyme assays.

LOX activity was measured following a modified method of Surrey (1963). The reaction mixtures contained 2.775 mL 100 mM phosphate buffer (pH 8), 25 μL linoleic acid substrate solution and 0.2 mL of the supernatant. The increase in absorbance at 234 nm due to the formation of conjugated diene was determined, and the activity of LOX was expressed on a fresh weight basis as U g^{-1}, where U = 0.01 $\Delta A_{234\ nm}$ per min.

PPO activity was assayed by the increase in absorbance at 420 nm according to Murr and Morris (1974), with some modifications. The activity of PPO was performed in mixture containing 2.8 mL 9 mM catechol and 0.2 mL of the supernatant. The enzyme activity was expressed on a fresh weight basis as U g^{-1}, where U = 0.01 $\Delta A_{420\ nm}$ per min.

POD activity was determined using the method of Kochba, Lavee, & Spiege, 1997. The reaction mixture consisted 2.4 mL 50 mM phosphate buffer (pH 6.8), 0.25 mL 0.16 M guaiacol, 0.25 mL 0.88 M H_2O_2 and 0.1 mL of the supernatant. The increase in absorbance at 470 nm was measured, and POD activity was expressed on a fresh weight basis as U g^{-1}, where U = 0.01 $\Delta A_{470\ nm}$ per min.

PAL activity was measured in accordance with a modified method of Assis, Maldonado, Muñoz, Escribano, and Merodio (2001). The supernatant (0.2 mL) was incubated with 1.8 mL of 200 mM borate buffer (pH 8.8) and 1 mL L-phenylalanine (0.6 mM) for 1 h at 37 °C. The activity of PAL was calculated from the change in absorbance at 290 nm caused by the formation of trans-cinnamate, and expressed on a fresh weight basis as U g^{-1}, where U = 0.01 $\Delta A_{290\ nm}$ per min.

CAT activity was assayed according to Dhindsa et al. (1981), with some modifications. The reaction mixtures contained 2.6 mL 50 mM phosphate buffer (pH 7.5), 0.3 mL 2 mM H_2O_2 and the supernatant. The activity of CAT was calculated from the change at 240 nm, and the activity of CAT was expressed on a fresh weight basis as U g^{-1}, where U = 0.01 $\Delta A_{240\ nm}$ per min.

APX activity was assayed by the decrease in absorbance at 290 nm following a modified method of Nakano and Asada (1989). The activity of APX was performed in mixture containing