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Mild concentration of ethanol in combination with ascorbic acid inhibits browning and maintains quality of fresh-cut lotus root

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ABSTRACT

Aqueous solutions of ethanol and ascorbic acid alone and in combination were compared to a commonly used sanitizer, sodium hypochlorite, and a leading commercial antibrowning agent containing calcium ascorbate (CA) for their efficacy to inhibit microbial growth and browning on fresh-cut lotus root. Fresh-cut lotus root slices were immersed in one of 7 treatment solutions including water (control), 30% ethanol (v/v), 30% ethanol (v/v) plus 30 g L^{-1} ascorbic acid (AA), 30% ethanol (v/v) plus 30 g L^{-1} CA, 30 g L^{-1} AA, 30 g L^{-1} CA and 100 mg L^{-1} sodium hypochlorite for 2 min, and then packaged in polyethylene bags and stored for 28 d at 4 °C. Packages were monitored for headspace gas composition, color, texture, aerobic mesophilic bacteria, and yeast and mold populations, electrolyte leakage and sensory attributes. The results indicate that all ethanol treatments, with and without added AA or CA, not only inhibited microbial growth, but also delayed browning more effectively than either AA or CA alone. The combined treatments of Ethanol 30% (v/v) along with 30 g L^{-1} AA or 30 g L^{-1} CA were even more effective than ethanol alone in maintaining quality of fresh-cut lotus slices during cold storage. Increasing ethanol concentration within the range of 5–30% (v/v) when accompanied by 30 g L^{-1} AA, decreased microbial populations and had little effect on quality maintenance of fresh-cut lotus root during cold storage. Ethanol concentrations of 20–30% (v/v) in conjunction with 30 g L^{-1} AA have the potential to inhibit browning and maintain quality of fresh-cut lotus root slices for more than 14 d when stored at 4 °C. This is the first use of ethanol as a dual control for both browning and microbial growth in fresh-cut lotus root.
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1. Introduction

Lotus root is a popular aquatic root vegetable world-wide because of its sensory attributes and nutritional value (Du et al., 2009; Xing et al., 2010). High levels of potassium, iron, copper, thiamine, riboflavin, pyridoxine, vitamin C, polyphenols and dietary fiber (USDA, 2015) confer many health benefits, such as improving blood circulation, digestion, heart health, mental clarity, mood, and immune function, and reducing anxiety, headache and blood pressure (Mukherjee et al., 2010; Sridhar and Bhat, 2007). Zhao et al. (2014) identified several alkaloids present in lotus root, which has been used traditionally along with the leaf seed and flower to treat many conditions including small pox, dysentery, cholera, cough, bleeding disorders, sunstroke, dizziness, cancer, hyperdipsia, strangury, vomiting, leprosy, skin diseases, anxiety disorders, and as a poison antidote (Mukherjee et al., 2009; Sridhar and Bhat, 2007; Yang et al., 2007). Additionally, lotus root has mucoprotective properties, which are beneficial in the treatment of indigestion and hemorrhoids (Chatterjee and Pakrashi, 1991; Mukherjee et al., 2009) and anti-diabetic and anti-inflammatory properties due to presence of a steroidal triterpenoid (Lee et al., 2001; Mukherjee et al., 2009). Lotus root has also been demonstrated to have anti-obesity, hepatoprotective, antipyretic, antioxidant, antibacterial, antifungal, antidiarrheal, and diuretic activities, as well as sedation effects (Hu and Skibsted, 2002; Mukherjee et al., 2009; Tsuruta et al., 2011; Yang et al., 2007; You et al., 2014).

Fresh lotus roots, which are harvested from bogs, are highly perishable and brown easily after fresh-cut processing due to increased respiration rate, oxidation by phenol metabolism-associated enzymes and microbial growth in the injured tissues (Ahvenainen, 1996; Hu et al., 2010; Jiang et al., 2014; Zhang et al., 2013). Improving quality and shelf-life of fresh-cut lotus root will

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reduce costs associated with losses and increase demand for this commodity. Browning is the main physiological disorder that results in product losses for fresh-cut lotus root. The enzyme activity that results in browning may additionally destroy some bioactive compounds, reducing the nutritional quality of lotus root. Many treatments have been researched and applied in attempt to inhibit browning and maintain quality of fresh-cut lotus root, including heat in conjunction with anaerobic conditions (Son et al., 2015), carbon monoxide (CO) fumigation (Zhang et al., 2013), hydrogen sulfide (H_2S) fumigation (Sun et al., 2015), chitosan-based coating (Xing et al., 2010), aqueous chlorine dioxide (ClO_2), ascorbic acid, erythorbic acid, citric acid, L-cysteine, $NaHSO_3$ and modified atmosphere (Du et al., 2009; Jiang et al., 2014; Lee and Eun, 1999).

All of these methods have some drawbacks. Storage of fresh-cut lotus root under anaerobic conditions would cause fermentation to occur in living tissues resulting in an unpleasant odor. Carbon monoxide and H_2S are poisonous gases, which are dangerous to work with and those studies were only conducted for seven and ten days, respectively, which may not have been long enough to determine whether the treatments resulted in tissue damage that shortened shelf life. Chitosan-based coating of vegetables is not desirable to consumers maintaining a vegetarian, vegan or kosher diet. Ascorbic acid and citric acid have limited effect as antimicrobials and antibrowning agents and L-cysteine imparts a bitter taste to the lotus root. Sodium bisulfite was banned from use on fresh fruit and vegetables in the United States in 1986, because of severe reactions in allergic individuals (U.S. FDA, 1986, 2011). Modified atmosphere packaging (MAP) is not in itself sufficient to prevent browning.

Ethanol is a potent antimicrobial agent commonly used in medical and biological sterilization of inanimate surfaces. Ethanol is a 'generally recognized as safe' (GRAS) substance, naturally found in plants, and at low concentrations causes little damage in fruit and vegetables (Hu et al., 2010; Janisiewicz and Conway, 2010). Additionally, several researchers have used ethanol vapors or dips to sanitize plant materials to inhibit decay (Candir et al., 2012; Lichter et al., 2002; Lurie et al., 2006) and growth of microbial populations including *Botrytis cinerea* (Chervin et al., 2009, 2005; Karabulut et al., 2004) and *E. coli* (Pinto et al., 2006) on grapes. Additionally, ethanol treatments have been demonstrated to retard tissue senescence and ripening, inhibit ethylene synthesis and action, decrease spoilage, and reduce chilling injury symptoms in a variety of fruit and vegetables including mango (Gutiérrez-Martínez et al., 2012; Plotto et al., 2006), apple (Bai et al., 2004), sweet cherry (Bai et al., 2011), oriental sweet melon (Liu et al., 2012), asparagus (Herppich et al., 2014), eggplant (Hu et al., 2010), and sunchoke (Wang et al., 2014). Ascorbic acid has been reported to effectively control enzymatic browning of fruit and vegetables (Du et al., 2009; Lee and Eun, 1999; Son et al., 2015). In our previous research we discovered that ethanol at low concentrations was able to control browning in apple slices. However, the use of ethanol to maintain microbiological and sensorial quality in fresh-cut lotus root is unknown. The objective of this study was to assess the effectiveness of low concentrations of ethanol in combination with other anti-browning agents and MAP to maintain color, texture, tissue integrity, and sensory attributes of and suppress microbial growth on fresh-cut lotus root slices stored at 4 °C.

2. Materials and methods

2.1. Plant material

Lotus roots (*Nelumbo nucifera* Gaertn.) were purchased from a grocery store in Silver Spring, MD, USA, transported to the laboratory and stored at 4 °C overnight, before processing. Samples were selected for uniformity of size and color, and absence of mechanical damage. Whole lotus roots were rinsed with tap water to remove soil and washed with sodium hypochlorite (NaOCl) solution (100 mg L^{-1} free chlorine, pH 6.5), which was prepared using Clorox (6% (v/v) sodium hypochlorite, Clorox Co., Oakland, CA) and the pH was adjusted using citric acid solution. Lotus roots were hand peeled, in one direction using a manual peeler to remove a minimal amount of surface tissue and then sliced 5 mm thick using a meat slicer (Berkel, Italy).

2.2. Experiment 1: assess efficacy of 30% ethanol treatments to inhibit browning and microbial growth of fresh-cut lotus root

Seven wash treatments were prepared with deionized water including: 1) non-chlorinated water, 2) 30% (v/v) ethanol (E), 3) 30 g L^{-1} ascorbic acid (AA), 4) 30 g L^{-1} NatureSeal® anti-browning agent with sole ingredient calcium ascorbate (CA) (NatureSeal Inc., USA), 5) 30% E + 30 g L^{-1} CA, 6) 30% E + 30 g L^{-1} AA, 7) sodium hypochlorite (NaOCl) wash solution (100 mg L^{-1} free chlorine, pH 6.5). Fresh-cut lotus root slices (3.4 kg) were submerged and manually agitated in 3 L of one of the 7 treatment solutions for 2 min. Washed samples (100 ± 2.0 g) for each treatment were packaged in polyethylene bags (22.5 cm × 17 cm, Pacific Southwest Container Inc., Modesto, CA, USA) with film oxygen transmission rate (OTR) of 21.4 pmol s^{-1} m^{-2} Pa^{-1} and stored at 4 °C. Package atmospheres, color, texture and microbial analyses were performed on days 1, 7, 14, 21, 28. Tissue electrolyte leakage and sensory evaluation were performed on day 28.

2.3. Experiment 2: effect of ethanol concentration

Lower concentrations of ethanol combined with ascorbic acid including: 5% E + 30 g L^{-1} AA, 10% E + 30 g L^{-1} AA, 20% E + 30 g L^{-1} AA, and 25% E + 30 g L^{-1} AA, were tested alongside 30% E + 30 g L^{-1} AA to determine whether they could inhibit browning and maintain quality of fresh-cut lotus root slices. Other than the wash treatments, all the processing steps were kept the same as in experiment 1. Package atmosphere, color and microbial analyses were performed on days 1, 7, and 14.

2.4. Quality evaluation

2.4.1. Package headspace gas composition

The package atmospheres were measured immediately upon removal of the samples from storage. Gas samples were withdrawn from the package by inserting the needle of a measuring assembly through a septum adhered to the packaging film and O2 and CO2 concentrations were determined using a gas analyzer (Check mate II, PBI Dansensor Co., Denmark).

2.4.2. Color assessment

The surface color of samples was measured with a colorimeter (Konica Minolta CR-400 Chroma Meter, Ramsey, NJ, USA). The instrument was calibrated with a white tile (Y = 94.0, x = 0.3130 and y = 0.3191). Measurements were taken for L^*, a^* and b^* values at 2 sites on each of 20 lotus slices for each treatment group. Color coordinates, a^* and b^*, were converted into hue angles [hue = $\tan^{-1}$(b/a)].

2.4.3. Texture analysis

Texture properties of the samples were assessed using a TA-XT2i texture analyzer (Texture Technologies Corp., Scarsdale, NY, USA) with the following parameters: load cell = 30 kg, probe = 4 mm diameter aluminum cylinder and test speed = 2 mm s^{-1}. The firmness was defined as the maximum force needed to puncture the lotus root slices to a depth of 3.5 mm. Measurements were

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