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 to 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 to 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.

Key words: fresh-cut lotus root, ethanol, ascorbic acid, anti-browning, antimicrobial, quality

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 & 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 & Bhat, 2007; Yang et al., 2007). Additionally, lotus root has mucoprotective properties, which are beneficial in the treatment of indigestion and hemorrhoids (Chatterjee & 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 & 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 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 (H₂S) fumigation (Sun et al., 2015), chitosan-based coating (Xing et al., 2010), aqueous chlorine dioxide (ClO₂), ascorbic acid, erythorbic acid, citric acid, *L*-cysteine, NaHSO₃ and modified atmosphere (Du et al., 2009; Jiang et al., 2014; Lee & 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 H₂S 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 & 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; Chervin et al., 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 & 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 m²Pa⁻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 [$\text{hue} = \tan^{-1}(b/a)$].

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 performed at 2 sites in the cortex of each fresh-cut lotus root slice. For each treatment, 5 slices were measured for each of 3-5 replicates.

2.4.4. Electrolyte leakage analysis

Tissue electrolyte leakage was measured following a modified procedure (Wang, Feng et al., 2004). For each package, a sample of fresh-cut lotus root (100±2 g) was submerged in 300 mL of deionized water for 30 min at 22°C. The electrolyte content of the solution was determined by measuring the electrical conductivity with a conductivity meter (model 135A, Orion Research, Inc., Beverly, MA, USA). Total electrolytes of the fresh-cut lotus root samples were determined after repeatedly freezing at -20 °C for 24 h and thawing at room temperature. Relative electrical conductivity (REC) was expressed as a ratio of fresh over total electrolytes.

2.4.5. Sensory evaluation

A six-member trained sensory panel conducted the visual and olfactory evaluation of the fresh-cut lotus root samples stored for 28 days. Overall visual quality was assessed with a 9-point hedonic scale where 9 = like extremely, 7 = like moderately, 5 = neither like nor dislike, 3 = dislike moderately, 1 = dislike extremely (Bai et al., 2004). Off-odor was evaluated on a scale of 1 to 5, where 1 = none, 3 = moderate and 5 = severe (Kou et al.,

2014b). The samples were coded with a random three-digit number to mask the treatment identity, in order to minimize subjectivity and were compared with freshly prepared samples.

2.5. Microbial assays

Microbial growth on the fresh-cut lotus root slices was assessed during each evaluation day. Each 25 ± 0.5 g sample was blended with 100 mL of sterile phosphate buffered saline (PBS, Fisher Scientific, Pittsburgh, PA, USA) using a stomacher bender (Model 80, Seward Medical, London, UK) for 2 min. The filtrate and its appropriate dilutions were logarithmically spread on agar plates with a Whitley automatic spiral plater (Wasp II, Don Whitley Scientific Ltd., West Yorkshire, UK). The aerobic mesophilic bacterial (AMB) population was determined by plating samples on tryptic soy agar (TSA, Difco, Detroit, MI, USA) and incubating at 28 °C. Yeast and mold (Y&M) enumeration was performed by plating on potato dextrose agar (PDA, Difco, Detroit, MI, USA) supplemented with 200 mg L⁻¹ chloramphenicol (Sigma-Aldrich, St Louis, MO) and incubating at room temperature (22°C). Microbial colonies were counted using a ProtoCOL automated colony counter (Synbiosis, Cambridge, UK) and reported as log CFU/g of tissue.

2.6. Statistical analysis

Package atmospheres, color, texture, and microbial enumeration data were analyzed as two-factor (treatment and storage duration) linear models using the PROC MIXED procedure (SAS Institute Inc 1999, Cary, NC). Assumptions of normality and variance homogeneity of the mixed model were checked and the variance grouping technique was used to correct for variance heterogeneity. When effects were statistically significant, means were compared using Sidak

adjusted p-values to maintain experiment-wise error ≤ 0.05 . For electrolyte leakage (REC) and sensory data, treatment differences were tested with Tukey-Kramer test, $\alpha=0.05$. Analysis of Variance and Pearson correlation analysis (SAS Version 9.2, SAS Institute, Inc.) were used to generate regression models and cross correlate among parameters: CO₂, O₂, Peak force, AMB, Y&M, L^* value, hue angle, REC, off-odor and overall visual quality. The data was reported as the mean of 3-5 replicates $\pm$ standard error (SE).

3. Results and Discussion

3.1. Experiment 1: Assess efficacy of 30% ethanol treatments for inhibition of browning and microbial growth

3.1.1. Package headspace gas composition

Headspace gas composition is an indication of the product's respiration rate (Kou et al., 2014a). Lower oxygen and higher carbon dioxide indicate a higher respiration rate which tends to accelerate quality degradation and shorten shelf life.

Fig. 1. Evolution of O₂ and CO₂ plotted as a function of storage time for fresh-cut lotus root slices (experiment 1). Data represent the mean $\pm$ S.E. (n=4).

Figure 1 shows the changes in O₂ and CO₂ partial pressures of fresh-cut lotus root slices stored at 4 °C for 28 d. There was no significant difference in gas atmospheres among water wash, 30 g L⁻¹ CA and NaOCl treatments during storage, while O₂ and CO₂ partial pressures for 30 g L⁻¹ AA treatment had a sharper decline and increase, respectively, during the last week of storage ($P < 0.01$). The O₂ partial pressure in treatments without ethanol declined sharply during the first 7 d (from 14.25-18.45 kPa to 0.02-0.53 kPa), then rose again slowly, but after 21 d declined again for some treatments. Conversely, the CO₂ partial pressure in treatments without ethanol increased sharply during the first 7 d (from 0.65-1.23 kPa to 4.28-6.25 kPa), then declined over the next 14 d, rising again during the last 7 d of storage. This pattern suggests that the samples of the four non-ethanol treatment groups had a high respiration rate and thus consumed the O₂ and produced a high concentration of CO₂ in the package during the first week after processing. There were no significant differences in O₂ or CO₂ partial pressures between 30% E + 30 g L⁻¹ AA and 30% E + 30 g L⁻¹ CA treatments during storage, but these 2 treatments had significantly higher and lower O₂ and CO₂ partial pressures, respectively, than other treatments ($P < 0.01$). Partial pressures of O₂ and CO₂ for 30% E treatment were next highest and lowest, respectively. Sample packages for treatments containing ethanol maintained O₂ (14.20-15.95 kPa) and CO₂ (0.65-2.23 kPa) partial pressures at more nearly a steady state than those of other treatments, which indicated a reduced respiration rate in these samples. Wang, Nie and Cantwell (2014) also observed significantly depressed respiration rate in sunchoke tuber slices treated with ethanol. Other researchers have noted reduced respiration rates in ethanol treated apple (Bai et al., 2004), eggplant slices (Hu et al., 2010), and broccoli florets (Asoda et al., 2009). Since ethanol treatment reduced microbial growth in all these instances, the reduced respiration rate may be attributable to the lower

microbial populations. However, ethanol has been shown to inhibit mitochondrial function and enzyme activity (Kern et al., 2009) and this is more likely to be the cause of the lower respiration rates observed in packages of ethanol treated lotus root slices.

3.1.2. Color changes

Color is the basis for sorting lotus root slices into commercial grades (Siomos et al., 2010; Xing et al., 2010). For fresh-cut lotus root slices, a lower L^* value indicates tissue darkening, increase in a^* value indicates increase in red/orange tissue discoloration and a greater hue angle indicates tissue color change from pale to red/brown (Plotto et al., 2006).

Fig. 2. Color changes of fresh-cut lotus root slices expressed as changes in L^* , a^* and $hue\ angle$ values during storage at 4 °C (experiment 1). Data represent the mean $\pm$ S.E. (n=4).

Values for L^* and hue angle decreased with increasing storage time (Fig. 2), while a^* value increased (Data not shown). Samples treated with 30 % E + 30 g L⁻¹ AA and 30 % E + 30 g L⁻¹ CA had the least change in L^* value, hue angle and a^* value from the initial fresh value and therefore the least browning. Samples treated with 30 % E only had the second least change.

These preceded 30 g L⁻¹ AA only, followed by water wash control and NaOCl, and 30 g L⁻¹ CA alone had the strongest browning. Samples treated with 30 % E + 30 g L⁻¹ AA and 30 % E + 30 g L⁻¹ CA did not differ significantly in their L^* values or a^* values and had only slightly lower a^* values than 30 % E treatment. Color values for these two treatments did not change significantly over time, from day 7 until the end of storage ($P < 0.01$). The hue angle for 30 % E + 3 % AA was significantly higher than that for 30 % E + 30 g L⁻¹ CA and for 30 % E ($P < 0.01$). There was no significant difference between the hue angle for 30 % E + 30 g L⁻¹ CA and for 30 % E alone. Water treatment, 100 mg L⁻¹ NaOCl solution and 30 g L⁻¹ AA treatments had similar L^* , hue angle and a^* values, all with large change over time, while 3 % CA had lower L^* value and hue angle and higher a^* value even than water for the entire storage duration ($P < 0.01$), indicating that this treatment is not suitable for lotus root slices and might exacerbate browning. In this study, samples treated with ethanol maintained desirable gas composition and pale color during the 28 day storage period, but for samples treated without ethanol, gas composition and color changed greatly (Fig. 1 & Fig. 2). Samples treated with 30 % E + 30 g L⁻¹ AA and 30 % E + 30 g L⁻¹ CA maintained more stable L^* values throughout storage than those in other treatment groups. These values maintained greater stability than those reported by other researchers who attempted to inhibit browning in fresh-cut lotus root (Du et al., 2009; Sun et al., 2015; Xing et al., 2010; Zhang et al., 2013). The efficacy of ethanol to maintain light color of lotus root slices is most likely due to the effect of ethanol on inhibiting the PPO enzyme. Reduced respiration rate and high O₂ and low CO₂ partial pressures in packages of fresh-cut lotus root could increase browning as a result of greater oxidation. However, the effect of ethanol on suppressing enzyme activity is consistent with reports of its retarding effects on respiration rate (through inhibiting

mitochondrial function), ethylene synthesis, ripening and senescence (Bai et al., 2004, 2011; Hu et al., 2010; Suzuki et al., 2004, 2005; Wang et al., 2014). Wang et al. (2014) found that ethanol fumigation or dipping treatment significantly delayed and reduced the respiration peak, and delayed the red discoloration of fresh-cut tubers, similar to our results (Fig.1 & Fig. 2). Color data indicated that inhibition of browning and reddening was dependent on ethanol concentration and treatment time (Fig. 6).

3.1.3. Texture analysis

Crispness is one of the important organoleptic quality attributes of fresh lotus root (Dong et al., 2013). The texture data generally shows a trend of declining firmness over time (Fig. 3).

Fig. 3. Firmness measurements of fresh-cut lotus root slices (experiment 1). Data represent the mean $\pm$ S.E. for maximum force (n=3-5).

Firmness values for 30 % E + 30 g L⁻¹ AA and 30 % E + 30 g L⁻¹ CA treatment groups were not significantly different from each other during the entire storage duration and were higher than for other treatments. These treatment groups were followed in firmness by 100 mg L⁻¹ chlorine and 30 % E ($P < 0.01$). The lowest firmness values were measured for samples treated with water, 30 g L⁻¹ CA and 30 g L⁻¹ AA with values for the 30 g L⁻¹ AA treatment group showing the steepest decline over time. There was large sample variation among the texture data. Similar to the research of Bai et al. (2004, 2011), ethanol was found to have some positive effect on maintaining firmness (Fig. 3). It may reduce the activity of cell wall degrading enzymes such as polygalacturonase (PG), cellulase, endoglucanase (Cx), β -galactosidase (β -GAL) and 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase) (Bai et al., 2011; Pesis et al., 1998; Plotto et al., 2006). However, based on the electrolyte leakage data, ethanol may have resulted in membrane degradation and its effect on firmness may be the result of tissue dehydration. Both AA and CA in combination with ethanol improved firmness and decreased electrolyte leakage compared to ethanol only treatment suggesting a role for AA in alleviating tissue injury.

3.1.4. Electrolyte leakage analysis

Table 1 shows the percent electrolyte leakage values for the fresh-cut lotus root slices after 28 d storage. Samples treated with ethanol and ethanol combinations had significantly higher relative electrical conductivities (REC) than others ($p < 0.05$) indicating that the ethanol may cause some tissue damage. Samples treated with 30 % E had the highest REC values (16.01 %), followed by 30 % E + 30 g L⁻¹ AA treatment (12.85 %) and 30 % E + 30 g L⁻¹ CA treatment (12.44 %).

Table 1. Electrolyte leakage expressed as the ratio of fresh conductivity over total conductivity for the same sample after repeated freezing and thawing (n-5).

Treatment	Visual Quality (1-9)	Off Odor (0-5)	Purchase Intent (1-5)
water	3.17 ± 0.79 b	3.27 ± 0.54 b	1.93 ± 0.04 b
30 % E	4.03 ± 0.57 b	4.20 ± 0.34 a	2.53 ± 0.03 b
30 g L ⁻¹ AA	3.77 ± 0.91 b	3.33 ± 0.57 b	2.20 ± 0.04 b
30 g L ⁻¹ CA	3.20 ± 0.83 b	3.40 ± 0.56 b	2.00 ± 0.04 b
30 % E + 30 g L ⁻¹ CA	6.67 ± 0.66 a	3.67 ± 0.38 ab	4.07 ± 0.03 a
30 % E + 30 g L ⁻¹ AA	6.50 ± 0.73 a	3.40 ± 0.48 b	3.87 ± 0.04 a
100 mg L ⁻¹ NaOCl	4.03 ± 0.67 b	3.30 ± 0.51 b	2.50 ± 0.04 b

Values followed by a common letter are not significantly different ($P > 0.05$).

High ethanol in vegetable or fruit tissue may cause accumulation of ethyl esters which degrade the plasma membrane, increasing ion leakage (Bai et al., 2011; Suzuki et al., 2005). In our research, the electrical conductivity for ethanol-containing treatments was higher than for no-ethanol treatments (Table 1). Electrolyte leakage results suggest that 30 % ethanol treatments may lead to cell membrane degradation. The added ascorbic acid and calcium ascorbate may have reduced the severity of the tissue damage caused by the ethanol.

3.1.5. Sensory evaluation

Table 2 shows the sensory evaluation scores for overall visual quality, off-odor and purchase intent for the fresh-cut lotus root slices after 28 d storage. Samples from all treatment

groups had off-odor after 28 d storage. An alcohol off-odor was detected upon opening samples treated with 30 % E, 30 g L⁻¹ CA, 30 % E + 30 g L⁻¹ CA and 30 % E + 30 g L⁻¹ AA. However, these samples had less decay smell. Overall visual quality for 30 % E + 30 g L⁻¹ CA and 30 % E + 30 g L⁻¹ AA treatment groups and purchase intent scores were significantly higher than the scores for other treatment groups ($p < 0.05$), and above 6.0 and 3.0, the limits for salability for visual quality and purchase intent, respectively, while other treatment groups were not salable.

Table 2. Visual quality, off odor, and purchase intent scores for fresh cut lotus root slices evaluated by 6 trained panelists on day 28 (n = 5).

treatment	Relative Electrical Conductivity (%)
water	5.48 ± 0.46 c
30 % E	16.01 ± 0.48 a
30 g L ⁻¹ AA	4.61 ± 0.45 c
30 g L ⁻¹ CA	5.55 ± 0.32 c
30 % E + 30 g L ⁻¹ CA	12.44 ± 0.30 b
30 % E + 30 g L ⁻¹ AA	12.85 ± 0.23 b
100 mg L ⁻¹ NaOCl	3.92 ± 0.26 c

Values followed by a common letter are not significantly different ($P > 0.05$).

In conclusion, 30 % E + 30 g L⁻¹ CA and 30 % E + 30 g L⁻¹ AA treatments can maintain the quality of fresh-cut lotus root, but samples treated in these solutions do have residual alcohol smell, although not as strong as those treated in 30 % E alone. However, upon opening of the bag, the odor soon dissipated so that no direct off-odor due to ethanol remained. Other researchers also reported ethanol off-odor in ethanol-treated fruit and fresh-cut produce when opening sealed packages (Bai et al., 2004, 2011; Plotto et al., 2006).

3.1.6. Microbial assays

Figure 4 shows an increase in microbial counts for all treatments over the storage period, with the exception of yeast and mold (Y&M) for 30 % ethanol which did not change significantly.

Fig. 4. Microbial growth (log₁₀ cfu/g) on fresh-cut lotus root slices stored in modified atmosphere packaging for 28 d at 4 °C (experiment 1). Data represent the mean ± S.E. (n= 4).

Treatments containing ethanol maintained significantly lower levels of microbes than treatments without ethanol throughout storage. The aerobic mesophilic bacterial counts (AMB) for 30 % E + 30 g L⁻¹ AA treatment were significant lower than for 30 % E + 30 g L⁻¹ CA treatment throughout storage time. However, Y&M counts were not significantly different for these same 2 treatments. Both treatments had lower AMB and Y&M counts than did 30 % E ($P < 0.05$). The AMB for the 3 ethanol-containing treatments grew slowly remaining below 6.5 log

until after 14 d, and then more rapidly from 14 to 21 d, but AMB counts for treatments without ethanol increased rapidly during the first week reaching more than 8.5 log by day 7. The Y&M counts for ethanol-containing treatments fluctuated within the range of 2.7 log to 5.2 log, but counts for no-ethanol treatments sharply increased during the first 7 d and then remained over 6.8 log until the end of storage. Combined, 30 % E and 30 g L⁻¹ AA or 30 g L⁻¹ CA have a bacteriostatic and fungistatic effect, but used separately, 30 g L⁻¹ AA and 30 g L⁻¹ CA have no inhibitory effect on microbes. Reduced effects on both AMB and Y&M growth inhibition were observed for 30 % E treatment ($P < 0.05$). Our observations that ethanol treatment inhibited microbial growth (Fig. 4 and Fig. 7) and limited decay are similar to the results reported by other researchers (Bai et al., 2004, 2011; Lurie et al., 2006; Plotto et al., 2006). Janisiewicz and Conway (2010) showed that a combination of ethanol dip and biocontrol was more effective to reduce decay of fresh produce than each individual treatment alone. Our research also found that a combination of ethanol and ascorbic acid or calcium ascorbate significantly improved inhibition of microbial growth compared to ethanol alone.

3.2. Experiment 2: Effect of ethanol concentration

The results from Experiment 1 indicated that two treatments, 30 % E + 30 g L⁻¹ AA and 30 % E + 30 g L⁻¹ CA, were more effective for inhibiting browning and maintaining quality of fresh-cut lotus root. These two treatments groups did not have significant differences for most analyses. Since the commercial browning agent, NatureSeal® containing the CA used in this experiment is a patented product, AA was chosen for further research. In order to find a lower effective concentration, five concentrations of ethanol: 5 %, 10 %, 20 %, 25 % and 30 % were

tested along with 30 g L⁻¹ AA to investigate their influence on package headspace gas composition, browning and microbial growth.

3.2.1. *Package headspace gas composition*

The O₂ and CO₂ partial pressures of fresh-cut lotus root slices treated with different concentrations of ethanol with 30 g L⁻¹ AA and stored for 7 and 14 d are shown in Fig. 5.

Fig. 5. Evolution of O₂ and CO₂ plotted as a function of storage time for fresh-cut lotus root slices (experiment 2). Data represent the mean $\pm$ S.E. (n=4). Different upper case letters indicate significant differences between days within a treatment. Different lower case letters indicate significant differences among treatments within a day.

Packages of samples treated with higher concentrations of ethanol: 20, 25 and 30 % did not have significantly different O₂ (16.70-17.42 kPa) or CO₂ (0.47-0.82 kPa) partial pressures from each other and did not have a significant change in O₂ or CO₂ partial pressures between 7 and 14 d, remaining significantly higher in O₂ and lower in CO₂ partial pressures at 14 d than packages of samples treated with 5 and 10 % ethanol ($P < 0.01$).

3.2.2. Color assessment

The color measurements for fresh-cut lotus root slices stored at 4 °C are shown in Fig. 6.

Fig. 6. Color measurements of fresh-cut lotus root slices subjected to different ethanol concentrations, expressed as L^* and hue angle values during storage at 4°C (experiment 2). Data represent the mean $\pm$ S.E. (n=3). Different upper case letters indicate significant differences among days within a treatment. Different lower case letters indicate significant differences among treatments within a day.

Lightness (L^*) values declined significantly during the first 7 d of storage but were not significantly affected by ethanol concentration. Lightness values for all concentrations except 5 % ethanol, declined between day 0 and 7 d, but did not change significantly from day 7 to day 14. L^* values for 5 % ethanol and 30 % ethanol were significantly different on day 7, but not on day 14. L^* values for 5 % ethanol were not significantly different from those for 10, 20 and 25 % ethanol treatments on any evaluation day ($P < 0.05$). Also, a^* values and hue angles for 5, 10, 20 and 25 % E + 30 g L⁻¹ AA treatments were not significantly different on any evaluation day, but a^* values were significantly higher and hue angles significantly lower for 30 % E + 30 g L⁻¹ AA treatment than for 5, 10 and 20 % E + 30 g L⁻¹ AA treatments on days 7 and 14 ($P < 0.05$). Lower concentrations of ethanol therefore had less overall change in hue angle than higher concentrations during this experiment. It was noted that lotus root slices had more reddish color on day 7 than on day 14 which is consistent with the higher a^* values and lower hue angles recorded on day 7 than on day 14. However, this is an unexpected result and may be caused by inconsistent quality of raw material used. While lotus roots were relatively fresh at the start of experiment 1, they had been stored for several weeks prior to experiment 2.

3.2.3. Microbial assays

Figure 7 shows that microbial populations of 5 % E + 30 g L⁻¹ AA and 10 % E + 30 g L⁻¹ AA treatment groups increased over storage time, those of 20 % E + 30 g L⁻¹ AA and 25 % E + 30 g L⁻¹ AA treatment groups did not change significantly over time and that of 30 % E + 30 g L⁻¹ AA actually decreased over time. Additionally, microbial populations were negatively correlated with alcohol concentration.

Fig. 7. Microbial growth ($\log_{10}$ cfu/g) on fresh-cut lotus root slices stored in modified atmosphere packaging for 28 d at 4 °C (experiment 2). Data represent the mean $\pm$ S.E. (n= 4). Different upper case letters indicate significant differences among days within a treatment. Different lower case letters indicate significant differences among treatments within a day.

While AMB populations were significantly lower for 30 % E + 30 g L⁻¹ AA than for 20 % E + 30 g L⁻¹ AA on day 7 and 14 or for 25 % E + 30 g L⁻¹ AA on day 14, there was no significant

difference between AMB populations for 20 % E + 30 g L⁻¹ AA and 25 % E + 30 g L⁻¹ AA on day 7 and 14, or between AMB populations for 25 % E + 30 g L⁻¹ AA and 30 % E + 30 g L⁻¹ AA on day 7. There was no significant difference among Y&M populations for 20, 25 and 30 % E treatments on day 7. However, Y&M populations were significantly lower for 30 % E + 30 g L⁻¹ AA than for 20 % E + 30 g L⁻¹ on day 14 ($P < 0.05$).

4. Conclusion

The majority of measured data in Experiment 1 followed a similar trend with treatments containing and lacking ethanol clearly divided into two groups. The treatment groups that lacked ethanol showed dramatic changes in O₂ and CO₂ gas, color, and microbial populations between day 0 and day 7, while those that contained ethanol maintained stable values of the same parameters. A combination of ethanol and ascorbic acid or calcium ascorbate was more effective to inhibit microbial growth and improve quality of fresh-cut lotus root slices than ethanol alone. Understanding the mechanisms by which these combined treatments achieve these beneficial results will require further investigation.

Experiment 2 investigated the effect of different ethanol concentrations with added ascorbic acid to control microbes and maintain the quality of fresh-cut lotus root during cold storage. Consistent with most concentration gradient research (Wang et al., 2014), the microbial growth inhibition was proportional to the increase in ethanol concentration. Statistical analysis showed that although there were significant differences between 20 % and 30 % ethanol treatments with added ascorbic acid, these were small compared to the differences between treatment with and without ethanol. In conclusion, 20 % - 30 % ethanol in combination with 30 g L⁻¹ ascorbic acid and modified atmosphere packaging is an economic, practical and environmentally acceptable

approach to inhibit browning and maintain quality of fresh-cut lotus root slices during cold storage.

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