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Hot water and ethanol treatments can effectively inhibit the discoloration of fresh-cut sunchoke (*Helianthus tuberosus* L.) tubers

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ABSTRACT

The main problem affecting the quality of fresh-cut sunchoke tubers is cut surface discoloration. Pre- and post-cutting hot water and ethanol treatments were evaluated for their potential to inhibit discoloration, color changes, and associated phenolic metabolism in tuber slices stored in air at 5 °C. Some of the treatments tested inhibited discoloration and changes in a^* and hue color values. Slices that were post-cut treated with hot water at 50 °C for 6–8 min or 55 °C for 3–4 min and pre-cut treated with water at 50 °C for 20–25 min maintained good color for 8–12 days at 5 °C. Post-cut ethanol fumigation (150–750 μL/L for 5 h at 5 °C) can prevent discoloration for 30 d at 5 °C. Post-cut dips with ethanol solutions (3, 5, 8 or 10% for 5 min) increased shelf-life twofold or longer compared to untreated slices. Ethanol fumigation retarded the onset of wound-induced respiration rates as well as reducing maximum rates. A post-cut 10% ethanol dip also reduced respiration rates and reduced PAL activity and total phenolics. Ethanol dips had no effect and hot water treatments had no persistent effect on microbial loads over 12 d.

1. Introduction

Sunchoke or Jerusalem artichoke (*Helianthus tuberosus* L.) is a plant native to North America and currently produced in Mexico, United States, and China (Kays and Nottingham, 2008). Sunchoke tubers have a light or dark brown skin with a white, crisp, sweet pulp and can be consumed raw or cooked. The tubers contain 14–19% inulins (Cummings et al., 1995), non-digestible oligosaccharides with numerous purported health benefits, including lowering blood glucose, maintaining lipid homeostasis, and increasing mineral bioavailability (Niness, 1999).

Depending on cultivar and maturity, tubers can be stored at 0–2 °C for 6 months or longer (Kays and Nottingham, 2008). Besides dehydration, decay and sprouting defects, there is depolymerization of the inulins with storage time and an increase in simple sugars (Saengthongpinit and Sajjaanantakul, 2005).

Fresh-cut products aim to provide safe, fresh, and convenient produce items while requiring less transport and storage space than the intact commodity (Barrett et al., 2010; Francis et al., 2012; Watada et al., 1996). Innovations in fresh-cut product offerings can contribute to increased consumption of fruits and vegetables. Sunchoke tubers have the potential to be processed into fresh-cut products, but the main problem limiting shelf-life is a red discoloration of the cut surfaces (Wang and Cantwell, unpublished).

Inhibiting discoloration of fresh cut products has been studied for a wide variety of fruit and vegetables. Many approaches to resolve this problem have been taken including choice of cultivar, low temperature, low oxygen, high carbon dioxide atmospheres (Aquino-Bolaños et al., 2000) or super high oxygen (Jacxsens et al., 2001), and chemical and controlled atmosphere combinations (Gorny et al., 2002; Lu et al., 2006; Ma et al., 2010; Oms-Oliu et al., 2006). However, many of these treatments may cause off-odors and off-flavors or the compounds may not be generally recognized as safe (FDA, 2014). As a safe method, 45–55 °C heat-shock treatments have been shown to prevent browning reactions and maintain texture in various vegetables and fruits (Loaiza-Velarde et al., 1997; López-López et al., 2013; Tsouvaltzis et al., 2011). Heat treatment protected fresh-cut lettuce against browning, helped retain greenness, and decreased production of phenolics when applied either before or after cutting (Loaiza-Velarde and Saltveit, 2001).

Ethanol treatment is another method that has been used to inhibit browning of fresh-cut produce. Exposing lettuce mid-ribs to vapors or aqueous solutions of *n*-alcohols inhibited wound-induced tissue browning (Choi et al., 2005). Control of superficial scald (skin browning) in Red Delicious apples was achieved by treating the fruit with ethanol, butan-1-ol, or propan-1-ol (Ghahramani et al.,

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2000). Fresh-cut broccoli treated with ethanol maintained better storage quality (Han et al., 2006). Ethanol treated apples slices maintained appearance longer and had lower respiration rates than untreated slices (Bai et al., 2004). Ethanol was also found to extend the storage life of fresh-cut mango (Plotto et al., 2006) and fresh-cut eggplant (Hu et al., 2010).

Biosynthesis, oxidation and polymerization of phenolic compounds are often associated with discoloration and other color changes (Hisaminato et al., 2001; Rhodes and Wooltorton, 1978). In some products, PAL is considered the first committed enzyme in the phenylpropanoid pathway that regulates its overall activity (Dixon and Paiva, 1995). López-Gálvez et al. (1996) demonstrated a high correlation between the activity of phenylalanine ammonia lyase (PAL, EC 4.3.1.5.) and the discoloration on intact and cut lettuce leaves. Aquino-Bolaños et al. (2000) also demonstrated a close relationship between phenolic metabolism and browning of cut jicama tissue. However in other roots crops such as potato, there were no clear relationships between PAL, phenolic metabolism and cut tissue browning (Cantos et al., 2002; Luna et al., 2012; Ma et al., 2010). For some products such as apples and potatoes, polyphenol oxidase (PPO, EC1.10.3.1) activity is considered rate limiting. In general the most important factors that determine the rate of enzymatic browning of many fruit and vegetables are the concentrations of phenolic compounds in the tissue, tissue pH, temperature, and oxygen availability (Martinez and Whitaker, 1995; Rhodes and Wooltorton, 1978).

Although hot water and ethanol treatment have been effectively used to extend the shelf-life of some fresh-cut products, their effects on the phenolic metabolism and discoloration of fresh-cut sunchoke are unknown. The objectives of this study were to investigate potential treatments to control the discoloration of fresh-cut sunchoke tubers. Hot water and ethanol treatments applied before and after cutting were studied for their impact on visual quality, discoloration, respiration rates, phenolic metabolism and microbiology of the fresh-cut slices.

2. Materials and methods

2.1. Plant material and preparation

Medium size (60–90 g) sunchoke tubers (cultivars unknown) were purchased periodically from a local wholesaler in Sacramento, CA from product grown in Washington and California. Tubers were packaged in bulk in unsealed polyethylene bags in carton boxes and stored at 5 °C at the Mann Lab until used. Tubers were sorted and very small or defective (damage, decay) tubers were discarded. The tubers were scrub-washed with potable water and then rinsed in 200 μL L^{-1} sodium hypochlorite solution for 5 min, drained and air-dried.

Cleaned tubers were placed into clean LDPE (low density polyethylene) bags and returned to 5 °C until cut the following day. The terminal ends and protuberances (daughter tubers) were removed and tubers were cut into 4–5 mm thick slices on a V-Slicer PRIMA Mandoline (Borner, Germany) at 5 °C.

2.2. Hot water treatments

Four experiments were conducted to evaluate hot water temperature and dip times. After preliminary testing, two experiments evaluated potentially useful hot water treatments applied before and after slicing. Tubers were brought to room temperature and treated in a mass of water to mass of tuber ratio of 20–1. Water temperatures were raised to 1–2 °C above target temperature, tubers or slices immersed and water temperature was kept within ±1 °C of the target temperature.

2.2.1. Post-cut hot water treatment

Prepared slices were immediately placed in the hot water bath for the designated temperature and time. The first experiment evaluated untreated slices (control), and slices treated at 50 °C for 5 or 10 min, 55 °C for 2.5 or 5 min, or 60 °C for 1 or 2 min. Slices were evaluated after 0, 2, 4, 6 and 8 d at 5 °C with 3 replicates per treatment. In the second experiment treatments were control, 50 °C for 6, 8 or 10 min, and 55 °C for 3, 4 or 5 min. After hot water treatment, slices were immediately rinsed in 50 μL L^{-1} sodium hypochlorite water (pH 7.0) at 5 °C, blot dried with a paper towel to remove excess moisture and placed into unsealed small LDPE bags (8–9 pieces per bag). The ends were folded over and bags were placed on plastic trays inside unsealed large polyethylene bags, slices were evaluated after 0, 4, 8 and 12 d at 5 °C with 3 replicates per treatment. Control slices were also rinsed in cold chlorinated water.

2.2.2. Pre-cut hot water treatment

Two experiments were conducted in which the tubers were treated with hot water before slicing. The tubers were warmed 4–6 h at room temperature and daughter tubers were removed before treatment. The first experiment included the control, hot water at 45 °C for 15 or 30 min, 50 °C for 7.5 or 15 min, or 55 °C for 5 or 10 min. In the second experiment longer treatment times were used with 50 °C water for 15, 20 or 25 min and 55 °C water for 10, 15 or 20 min. The center of the tubers heated to 1–3 °C below the target temperature as measured by HI145 T-shaped thermometer (Hanna Instrument). After treatment, tubers were blotted dry, immediately cut into slices, placed in the unsealed LDPE bags and stored at 5 °C. Slices were evaluated after 0, 4, 8, and 12 d with 3 replicates per treatment.

2.3. Ethanol treatment

Three experiments were conducted in which ethanol vapor was applied before or after slicing or as a post-cutting dip solution.

2.3.1. Post-cut ethanol fumigation treatment

Sunchoke slices (about 70 at 7 g each) were placed without overlap on 2 lower layers of a plastic mesh grid in a closed 26 L polycarbonate chamber. A small fan for circulation and one absorbent gauze with different amounts of ethanol (0, 150, 300, 450, 600, 750 or 900 μL/L) were placed on the upper layer of the plastic grid. After fumigation for 5 h at 5 °C, the slices were then removed and directly placed in small unsealed LDPE bags and 3 replicates per treatment were evaluated after 0, 2, 4, 6, 8, 30, and 34 d at 5 °C.

2.3.2. Pre-cut ethanol fumigation treatment

The intact tubers were fumigated in the same way as described for post-cut ethanol treatment with 0, 300, or 600 μL/L ethanol for 5, 10, or 15 h at 5 °C. The slices were then prepared as previously described, packaged and 3 replicates per treatment were evaluated after 0, 4, 8, and 12 days at 5 °C.

2.3.3. Post-cut ethanol dipping treatment

Sunchoke slices were immersed in 0, 3, 5, 8, or 10% ethanol solutions for 5 min, blotted dry, placed in plastic bags and stored at 5 °C. Visual quality and color values were measured every 3 d until the ethanol treated slices began to redden.

2.4. Quality and shelf-life evaluation

The rating scales were developed by all three authors, but usually applied solely by the first author. Overall visual quality was evaluated by an experienced operator on a 9–1 scale, where 9 = excellent, fresh cut, no defects, 7 = good, minor defects, 5 = fair, moderate defects, 3 = poor, major defects, 1 = unusable. A

score of 6 was considered the limit of salability and shelf-life was defined at the days required to reach a score of 6. Red discoloration was evaluated on a scale of 1–5, where 1 = none, 2 = slight, 3 = moderate, 4 = severe, and 5 = extreme browning. Some of the heat treatments caused a yellowing or darkening that was distinct from the red discoloration; this damage discoloration was also evaluated on a 1–5 scale. Dehydration, translucency, and macroscopic decay were evaluated on scales of 1–5, where 1 = none, 2 = slight (up to 5% surface affected), 3 = moderate (5–20% surface affected), 4 = moderately severe (20–50%), and 5 = extreme (>50% surface affected).

2.5. Color

CIE $L^*a^*b^*$ values were determined with a Minolta Chroma Meter (Model CR-200/300, Minolta, Ramsey, N.J.) with illuminant A and a 10° viewing angle and calibrated on a white reference tile ($L^* = 97.95$, $a^* = -0.39$, $b^* = 2.00$). Color values were measured on 3 replicates of 8 pieces each per treatment. Chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) and Hue ($h^\circ = \tan^{-1}(b^*/a^*)$) were calculated. The correlations between visually scored discoloration scores and L^*, a^*, b^*, chroma, and hue values were 0.480, 0.880, 0.679, 0.802 and 0.922, respectively. Because hue and a^* color values were better related with discoloration scores, these values are reported.

2.6. Respiration

Respiration rates of slices were measured in the second ethanol fumigation and dip experiment. About 100 g of slices were placed in chambers through which humidified air (~90–95%) flowed at rates to obtain CO_2 concentrations between 0.25 and 0.5%. Respiration was determined by taking 1 mL samples from the outlet streams of the containers, and CO_2 was determined by infrared analysis (model PIR-2000, Horiba, Japan). Calculations were based on difference between inlet and outlet concentrations at atmospheric pressure (102 kPa, 50 °C) and CO_2 production rates were expressed on a fresh weight basis as $\mu L\,kg^{-1}\,s^{-1}$.

2.7. PAL enzyme activity

Phenylalanine ammonia lyase (PAL) activity was determined from 4 g finely chopped sample (without peel) placed into a plastic test tube on ice containing 0.4 g soluble polyvinylpolypyrrolidone (PVP) and frozen at −80 °C until analyzed. For analysis (Ke and Saltveit, 1986), 16 mL of 50 mM borate buffer (pH 8.5) (w/v) 400 μL/1000 mL 2-mercaptoethanol was added, homogenized, filtered on ice, centrifuged at 15,000 × *g* for 20 min. Two 2 tubes of 5 mL of supernatant were heated at 40 °C for 5 min, 0.55 mL of 100 mM L-phenylalanine was added to 1 tube and 0.55 mL water (blank) was added to the other tube. After mixing, absorbance was measured at 290 nm and tubes incubated at 40 °C for 1 h and absorbance measured again. One unit of PAL activity corresponded to the formation of 1 μmol of cinnamic acid in 1 h.

2.8. PPO enzyme activity

Polyphenol oxidase (PPO) activity was measured from 4 g finely chopped sample (without peel) weighed into a plastic test tube on ice containing 0.4 g insoluble PVP and frozen at −80 °C until analyzed. For analysis (Siriphanich and Kader, 1985), 16 mL phosphate buffer (50 mM pH 6.2) was added and sample homogenized. The homogenate was filtered through cheesecloth, the filtrate centrifuged at 12,000 × *g* for 20 min at 4 °C and the supernatant used for determination of PPO. To 933 μL of enzyme extract was added 67 μL caffeic acid (0.18 g in 10 mL ethanol) and the increase in absorbance was measured at 420 nm 1 min after adding the enzyme extract.

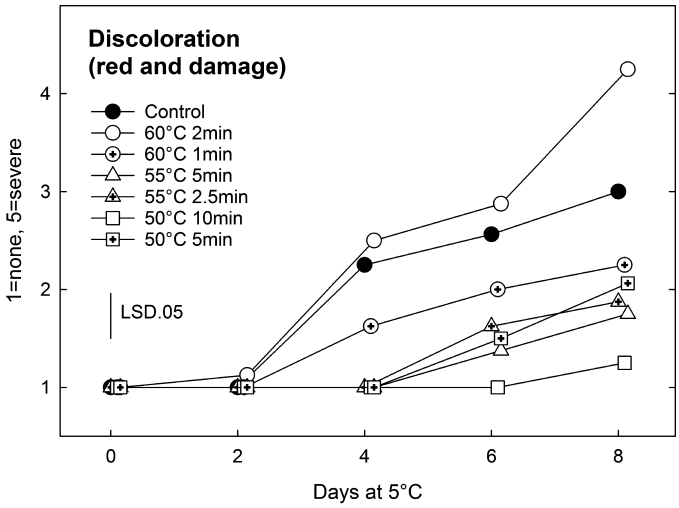

Fig. 1. Red discoloration of untreated or post-cut hot water (50 °C for 5 or 10 min, 55 °C for 2.5 or 5 min, 60 °C for 1 or 2 min) treated sunchoke slices stored at 5 °C. Data are means of 3 replicates of 8 slices each ± standard deviation. Scores of 1 and 5 indicate no discoloration and extreme browning, respectively.

One unit of PPO activity was defined as the amount of the enzyme that produced an increase of 0.1 absorbance units in 1 min.

2.9. Phenolics

The concentration of total phenolics was determined by a Folin–Ciocalteu method described by Singleton and Rossi (1965). Four grams of finely chopped tissue were frozen at −80 °C until homogenizing with 15 mL 80% ethanol. Reagent A was 2.7% sodium potassium tetrahydrated tartrate, Reagent B was 2.0% sodium carbonate (w/v) in 0.1 N sodium hydroxide, Reagent C was 1 volumetric part of Reagent A plus 98 parts of Reagent B (prepared at time of analysis), and Reagent D was 1 part of commercial Folin–Ciocalteu reagent and 1 part water (prepared at time of analysis). For analysis, 0.25 mL of filtered phenolic extract plus 2.5 mL Reagent C was let stand 10 min, 0.25 mL Reagent D was added and agitated and absorbance was measured at 660 nm after 30–60 min. Calculations were based on a standard curve of *p*-coumaric acid.

2.10. Microbiology

Microbiological examination was done in a separate experiment in which aerobic plate counts were enumerated from slices stored for 0, 6, and 12 d at 5 °C. Treatments were control (untreated), post-cut ethanol (300 μL/L 5 h), pre-cut ethanol (750 μL/L 15 h), post-cut hot water (50 °C 8 min) and pre-cut hot water (50 °C, 25 min). Samples of 25 g were homogenized in 225 mL distilled water. Total aerobic plate counts were determined from a dilution series for each treatment using SMA agar and incubating at 37 °C for 24 h (BAM, 1984).

2.11. Statistics

Experiments were conducted in a completely randomized design with a minimum of 3 replicates per treatment (1 replicate = 8–9 pieces). Data were calculated as averages ± standard deviations or analyzed by ANOVA with mean separation by LSD.05.

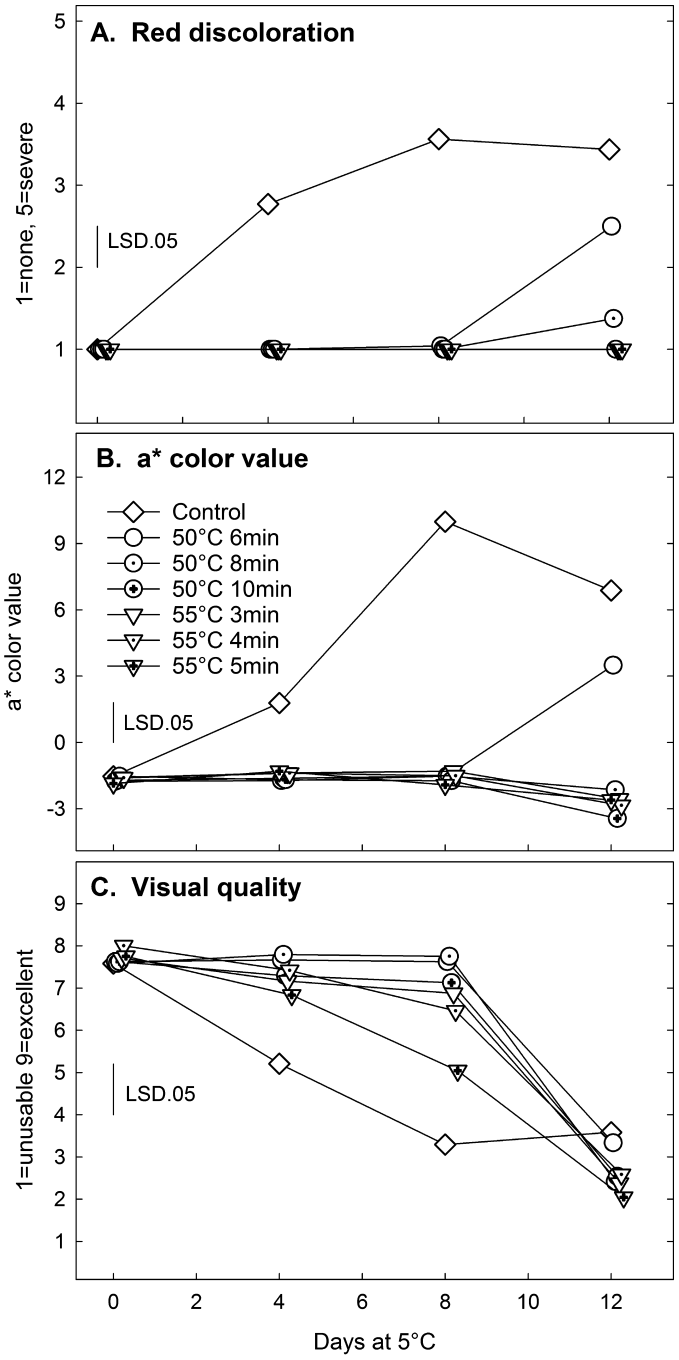

Fig. 2. Red discoloration (A), a^* color value (B), and overall visual quality (C) of untreated or post-cut hot water (50 °C for 6, 8 or 10 min; 55 °C for 3, 4 or 5 min) treated sunchoke slices stored at 5 °C. Data are means of 3 replicates of 8 slices each with mean separation by LSD.05.

3. Results and discussion

3.1. Hot water treatments and discoloration

Untreated fresh-cut sunchoke tubers show a red discoloration on their cut surfaces. Post-cut heat treatment can effectively prevent or retard discoloration of sunchoke slices (Fig. 1). Except for the 60 °C for 2 min treatment which damaged the tissues, all other post-cut heat treatments reduced discoloration to a score of 2 (slight) or less over 8 d at 5 °C (Fig. 1). The control slices had reddened by 2 d after cutting and discoloration increased to a moderate to severe rating (3–4) by day 8 (Fig. 1). The results are similar to

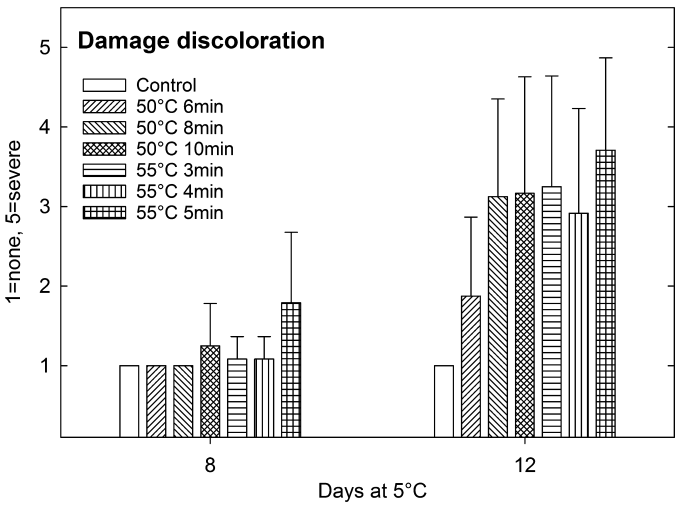

Fig. 3. Damage discoloration (yellowing, blackening) induced by post cut hot water treatments (see Fig. 2) on sunchoke slices after 8 and 12 d at 5 °C. Data means of three replicates of 8 slices each ± standard deviation.

previous studies using heat shock treatments to prevent browning reactions (Loaiza-Velarde et al., 1997; Loaiza-Velarde and Saltveit, 2001; Tsouvaltzis et al., 2011).

Some of the hot water treatments caused tissue softening and distinct color changes called damage discoloration (yellowing or blackening). On day 0, the slices treated at 60 °C for 2 min were more translucent than others, and from day 2 their cut surfaces partially browned and gradually darkened, indicating a heat treatment not tolerated by the product. However, 60 °C for 1 min resulted in slices that began to show red discoloration, indicating an ineffective treatment (Fig. 1).

The effect of post-cut hot water treatment on fresh-cut sunchoke was further confirmed in the second experiment. The untreated slices (control) had rapid discoloration, while discoloration did not change significantly in the treated slices for 8 days (Fig. 2A). This visual scoring of discoloration score corresponded well with the changes in a^* (Fig. 2B) and hue color values (data not shown). Although slices treated with 55 °C for 4 or 5 min and 50 °C for 10 min did not have red discoloration, they began to show heat damage by day 8.

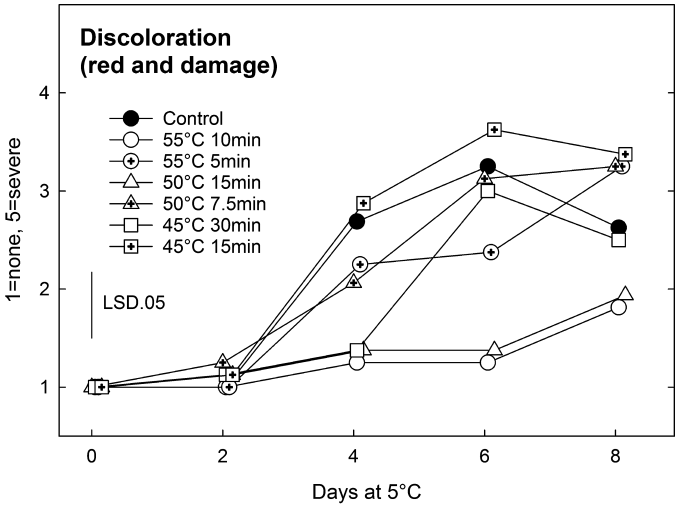

Fig. 4. Red discoloration of fresh-cut sunchoke stored at 5 °C. Slices were prepared from untreated or pre-cut hot water (45 °C for 15 or 30 min, 50 °C for 7.5 or 15 min, and 55 °C for 5 or 10 min) treated tubers. Data are means of 3 replicates of 8 slices each ± standard deviation.

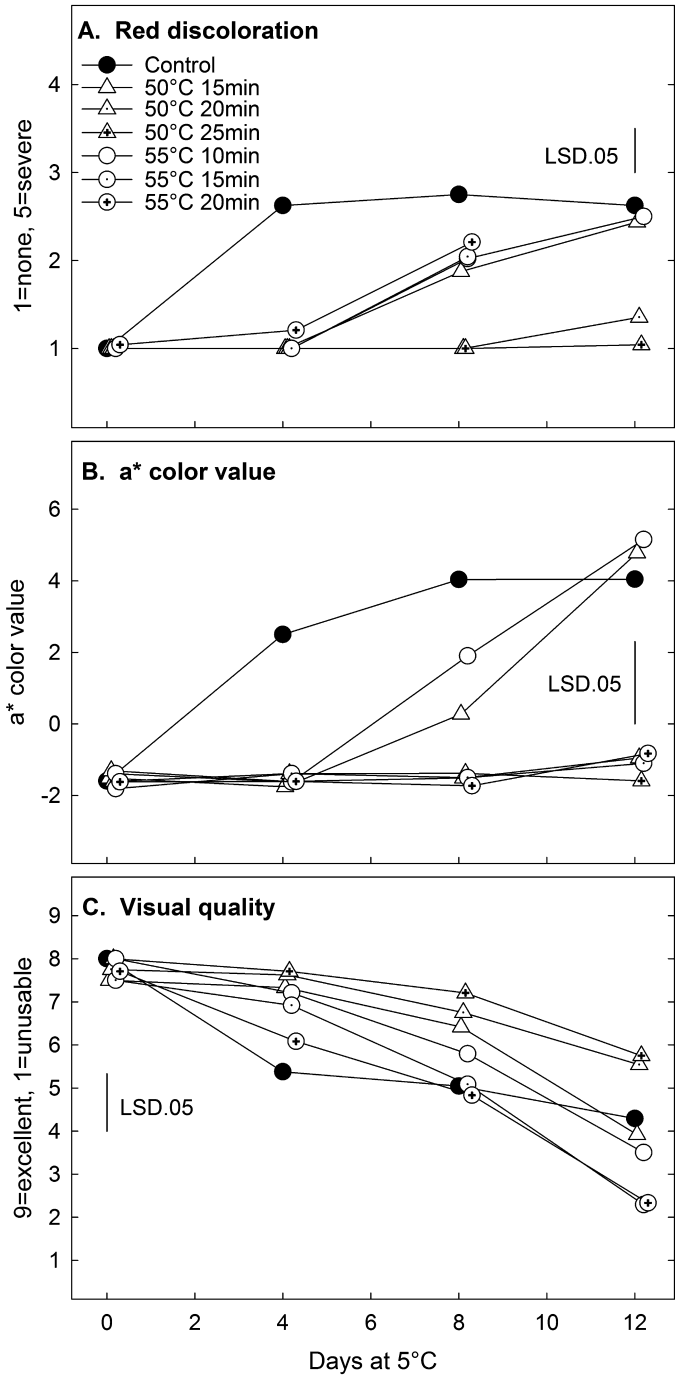

Fig. 5. Red discoloration (A), a^* color value (B), and overall visual quality (C) of fresh-cut sunchoke slices stored 12 days at 5 °C. Slices were prepared from untreated or pre-cut hot water (50 °C for 15, 20 or 25 min; 55 °C for 10, 15 or 20 min; 55 °C for 10, 15 or 20 min) treated tubers. Data are means of 3 replicates of 8 slices each with mean separation by LSD.05.

Changes in visual quality scores of the slices were mostly due to changes in red discoloration (Fig. 2C). Control slices were below the limit of marketability by day 4 (visual quality score less than 6). The slices treated with 55 °C for 5 min did not reach a score below 6 until day 8.

Overheating induced damage on the surface and also caused off-flavors and tissue softening. Hot water treatment with 55 °C for 5 min had significantly higher damage scores than other treatment by day 8 (Fig. 3), and 50 °C for 8 or 10 min and 55 °C for 3, 4 or 5 min had similar damage scores by day 12.

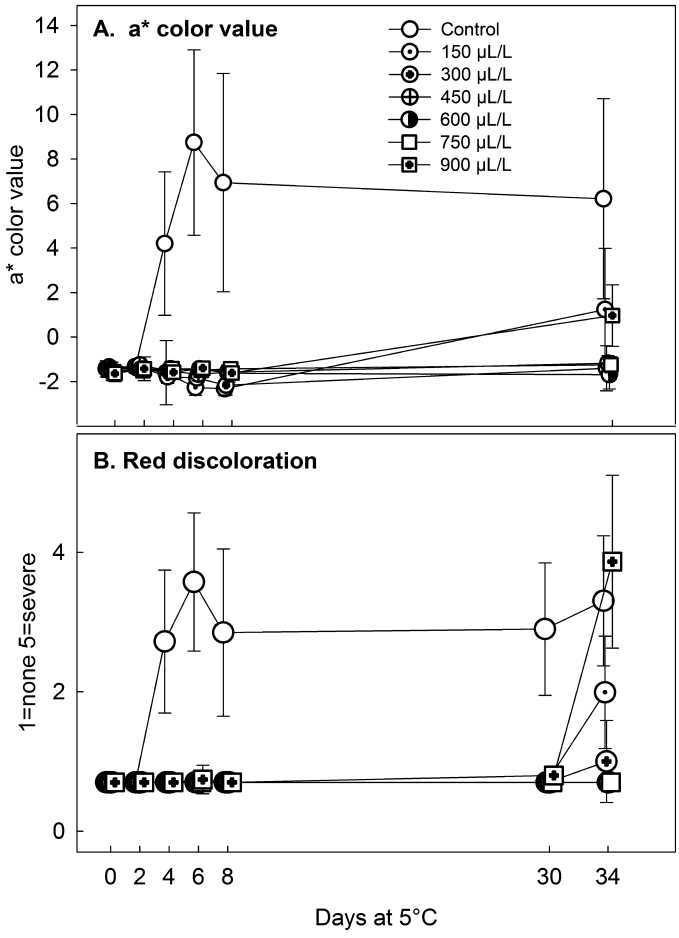

Fig. 6. The a^* color value (A) and red discoloration (B) of fresh-cut sunchoke stored for 34 days at 5 °C. Slices were untreated or post-cut ethanol fumigated (5 h with 150, 300, 450, 600, 750 or 900 µL/L). Data are means of 3 replicates of 8 slices each ± standard deviation.

Pre-cut hot water treatment also significantly inhibited the red discoloration of slices. The 50 °C for 15 min and 55 °C for 10 min treatments significantly reduced discoloration from day 4 to day 8 (Fig. 4). The 45 °C for 15 min, 50 °C for 7 min and 55 °C for 5 min were similar to or had more discoloration than untreated slices. Results reported here with hot water treatments are similar to other reports in which treatment-time combinations can be identified that reduce browning but do not cause injury. Loaiza-Velarde et al. (2003) found that 50 °C water for 90 s significantly reduced browning of cut celery mainly by reducing PAL activity. A 50 °C for 10 min hot water treatment combined with a delay in cutting retarded discoloration in cut potato (Tsouvaltzis et al., 2011).

Longer pre-cut heating periods were used to prevent discoloration (Fig. 5). The pre-cut 50 °C for 20 or 25 min were the two most suitable treatments tested, maintaining acceptable color (Fig. 5A and B) and visual quality (Fig. 5C) up to 12 d at 5 °C. Similar to post-cut experiment, the color changes observed with red discoloration were best represented by a^* (Fig. 5B) and hue color values (data not shown). In other research, hue values were closely related to discoloration of cut jicama roots (Aquino-Bolaños et al., 2000) and potato tubers (Ma et al., 2010) while L^* was most closely related to browning rate of fresh cut apples (Luo et al., 2011).

3.2. Ethanol treatments and discoloration

Post-cut ethanol fumigation showed promising effects on inhibiting discoloration for extended storage (Fig. 6). Untreated

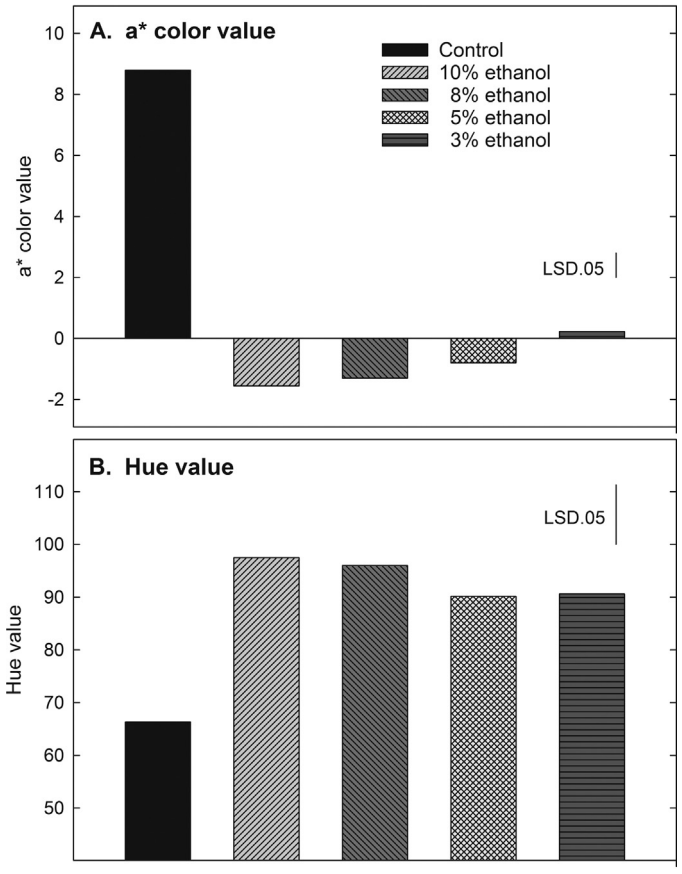

Fig. 7. The a^* (A) and hue (B) color values of fresh-cut sunchoke stored 15 d at 5 °C. Slices were untreated or post-cut ethanol (3, 5, 8 or 10% for 5 min) dip treated. Data are means of 3 replicates of 8 slices each ± standard deviation.

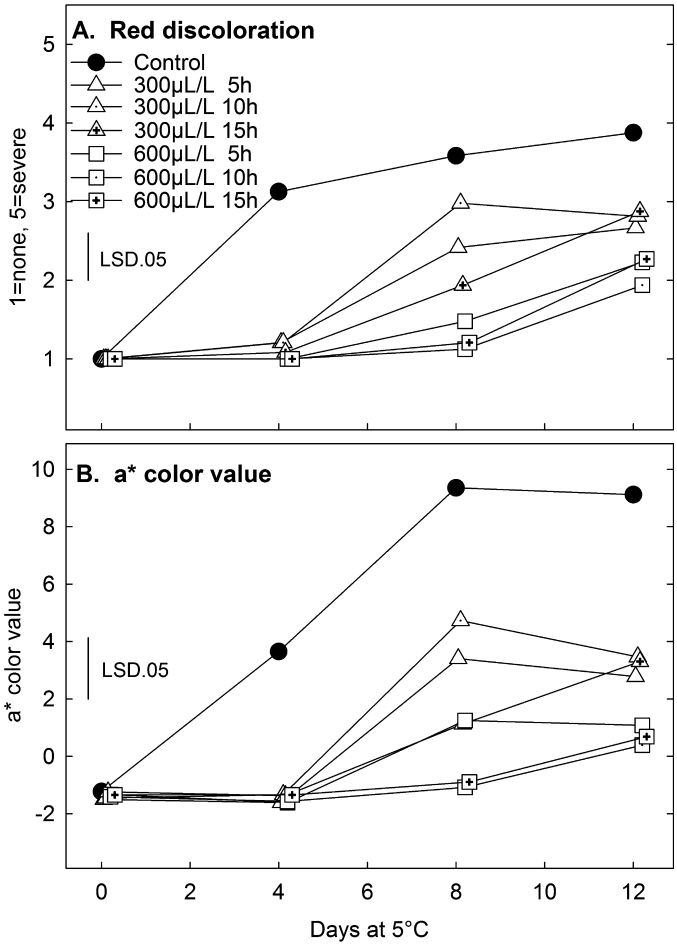

Fig. 8. Red discoloration (A) and a^* color value (B) of fresh-cut sunchoke stored 12 days at 5 °C. Slices were prepared from untreated or pre-cut ethanol fumigated (300 or 600 μL/L for 5, 10 or 15 h) tubers. Data are means of 3 replicates of 8 slices each with mean separation by LSD.05.

slices showed maximum discoloration by day 6, while many of the ethanol treated (450, 600, 750, and 900 μL/L) slices retained good visual quality for about 30 d. By day 34, the 150 and 300 μL/L ethanol treated slices turned red (Fig. 6A) and the 900 μL/L treated slices turned brown, which was considered ethanol overdose damage. When the slices were treated with 900 μL/L ethanol, the peels of the slices had visibly browned on day 0 but the cut surface did not became brown until day 34, indicating differences in tissue sensitivity to ethanol. Ethanol appeared to have no effect on the crispness or flavor of sunchoke slices.

In a post-cut ethanol dip experiment, the inhibitory effect of ethanol on red discoloration was again demonstrated. By day 15, 3% ethanol treated slices were slightly red, but the 5, 8 and 10% ethanol dipped slices retained the original color as reflected by the differences in a^* and hue color values (Fig. 7A and B). The higher a^* and lower hue color value indicated severe red discoloration.

As in the pre-cut hot water treatment, pre-cut ethanol treatment also delayed the red discoloration of fresh-cut tubers. The inhibitory effect was related to the ethanol dosage. In ethanol treated tissue, any discoloration was due to reddening of the cut surface (Fig. 8) with none of the damage discoloration observed in some of the heat treatments except for the highest ethanol concentration (900 μL/L). Slices treated with 600 μL/L ethanol maintained acceptable color to day 8, and red discoloration was very low (5% surface affected). The slices that were pretreated with 300 μL/L for 5 or 10 h reached slight or moderate discoloration by day 8 (Fig. 8A and B). The red discoloration a^* color data indicated that inhibition of reddening was dependent on ethanol concentration and time. For a given treatment time, the 600 μL/L ethanol treatment was clearly more effective than the 300 μL/L. Ethanol treatments affected the onset of red discoloration but not maximum scores or a^* color values. Bai et al. (2004) reported that ethanol treated apple slices retained visual acceptance for longer than control slices. Ethanol vapor treatments were also found to extend the shelf-life of fresh-cut mango by reducing discoloration (Plotto et al., 2006).

3.3. Ethanol treatments and respiration

Fresh-cut processing typically results in an increase in respiration rates (Surjadinata and Cisneros-Zevallos, 2003; Watada et al., 1996). There were large differences in the respiration patterns of untreated and ethanol treated (750 μL/L pre-cut for 15 h and 300 μL/L post-cut for 5 h) sunchoke slices (Fig. 9A). Ethanol treatments significantly inhibited the respiration rates after cutting. Untreated tissue reached maximum CO_2 production rates by day 5 (19.0 μL kg^{-1} s^{-1}) while maximum rates of precut ethanol treated slices were lower (11.7 μL kg^{-1} s^{-1}) and occurred on day 8, a reduction of about 38%. The maximum rate of post-cut ethanol treated tissue was even lower (8.0 μL CO_2/g-h) on day 11. The more rapid increase and higher rates of respiration were also associated with more rapid red discoloration (Fig. 9B and C). Respiration rates were reduced by ethanol treatment in sliced apples (Bai et al., 2004), fresh-cut eggplant (Hu et al., 2010), but not in fresh-cut mango (Plotto et al., 2006). Ethanol treatments suppressed ethylene production and respiration rates in broccoli florets (Asoda et al., 2009). Ethanol treatments were shown to reduce respiration rates and

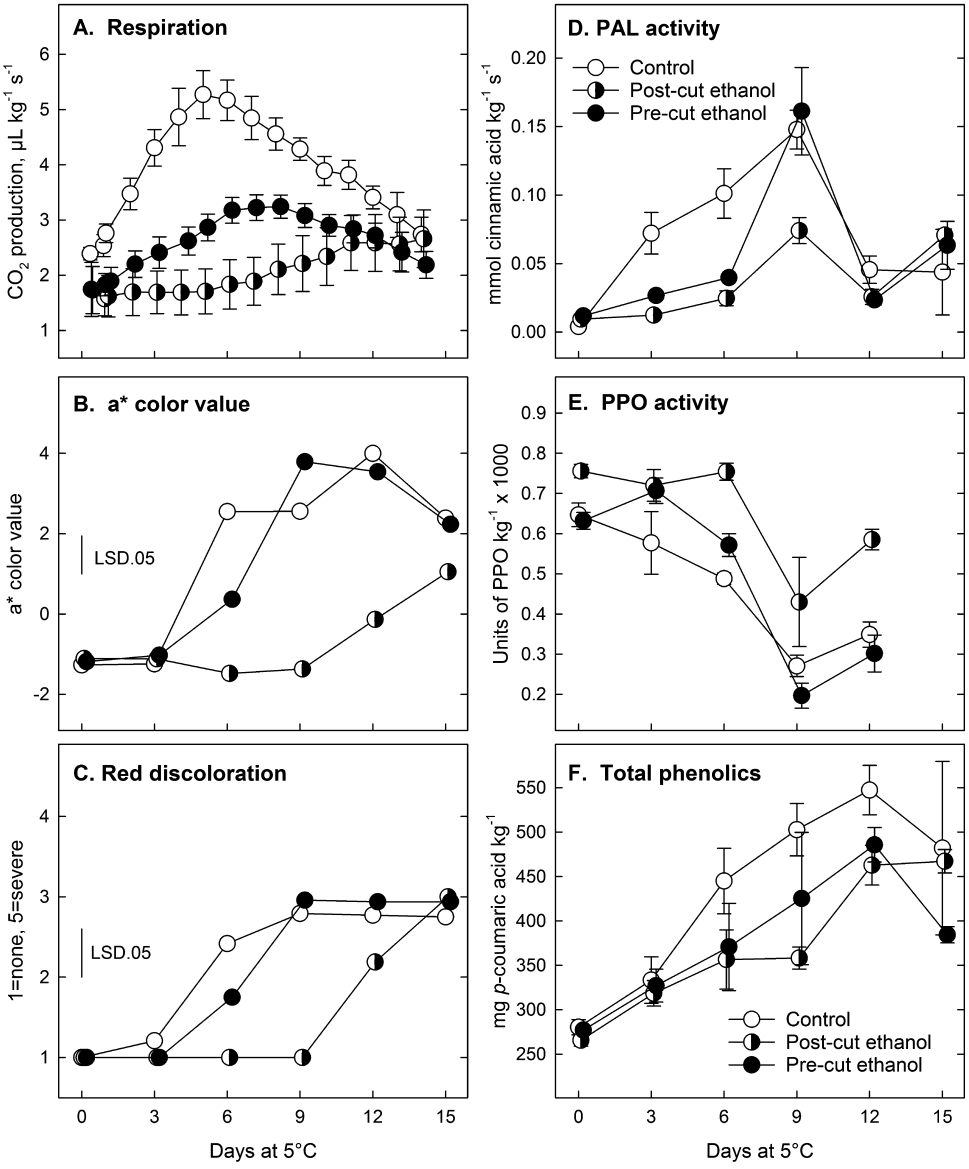

Fig. 9. Respiration rate (A), a^* color value (B), red discoloration (C), PAL enzyme activity (D), PPO enzyme activity (E), and total phenolic content (F) of untreated, post-cut ethanol treated (300 µL/L for 5 h) or pre-cut ethanol treated (750 µL/L for 15 h) sunchoke slices stored at 5 °C. Data are means of 3 replicates of 8 slices each ± standard deviation or mean separation by LSD.05.

ATP production of isolated mitochondria in *Euphorbia heterophylla* sprouts (Kern et al., 2009). Reduced respiratory activity and associated reduced energy production were clearly associated with reduced discoloration in sunchoke slices.

The time courses of respiration and red discoloration (Fig. 9A and C) clearly show that respiration rates changed before discoloration changes occurred. In control slices, respiration increased continuously from day 0, while discoloration began after 3 d. No red discoloration or changes in a^* values (Fig. 9B and C) were observed until 9 d in the post-cut 300 µL/L ethanol treatment.

3.4. Ethanol treatments and PAL, PPO and phenolics

Differences in PAL activity (Fig. 9D) were related to differences in the onset of red discoloration (Fig. 9A and B). The pre- and post-cut ethanol treatments significantly reduced PAL activity at day 3 and 6. The post-cut ethanol treatments resulted in the lowest PAL activity by day 9 which corresponded to no red discoloration or color change (Fig. 9B and C). No significant difference was detected between control and pre-cut ethanol treatment by day 9. These results are consistent with previous research by Saltveit (2000) in which heat shock treatment of iceberg lettuce leaves prevented an increase in PAL activity whether applied 4 h before or 2 h after cutting. PPO activities were, with some exceptions, similar among the 3 treatments, but variable over the 15 d storage period (Fig. 9E). The pattern of changes in phenolic compounds followed that of the onset of red discoloration (Fig. 9F). The ethanol treated tissues accumulated lower concentrations than untreated tuber slices, with concentrations increasing in all treatments up to day 12.

Post-cut ethanol dip treatment (10% ethanol for 5 min) was compared to untreated tissue for red discoloration, respiration rates, PAL activity, and total phenolic concentration (Fig. 10). The 10% ethanol dip completely inhibited discoloration of fresh-cut sunchoke and maintained low respiration rates over the 12 d period at 5 °C. Respiration rates of untreated slices were higher than in the previous test (Fig. 9), and this was probably related to the fact that tubers had begun sprouting in storage. As before, increases in respiration rates preceded the increases in red discoloration. Post-cut

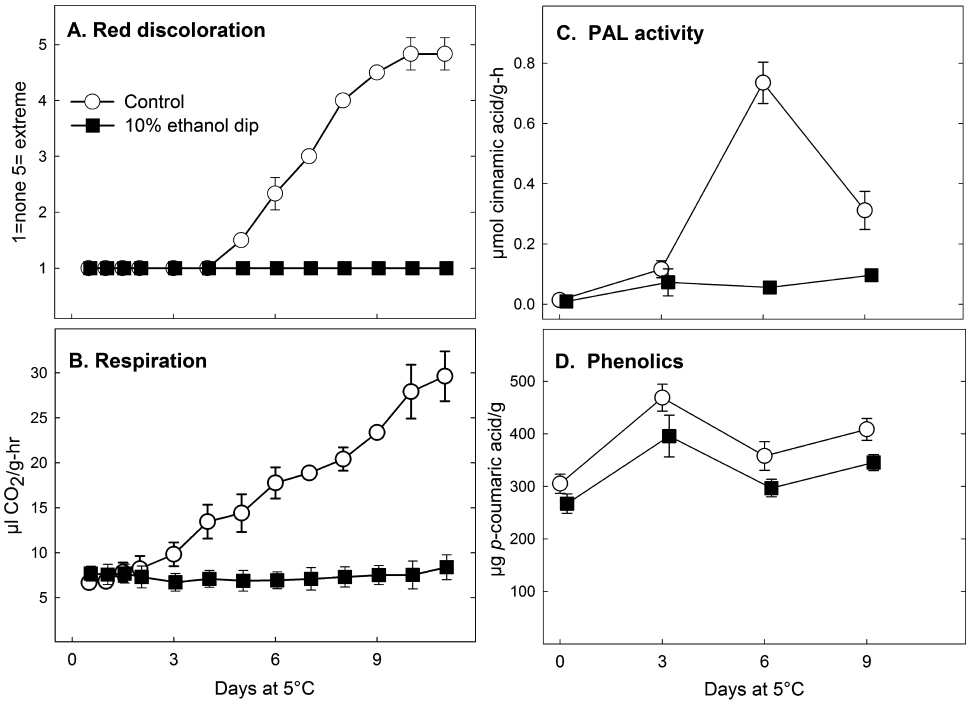

Fig. 10. Red discoloration (A), respiration rate (B), PAL activity (C) and total phenolic content (D) of untreated or post-cut 10% ethanol dip treated sunchoke slices stored at 5 °C. Data are means of 3 replicates ± standard deviation or with mean separation by LSD.05.

ethanol dip also prevented the increase in PAL activity observed in the untreated slices (Fig. 10C). PPO activities were similar between treatments and relatively stable over 9 d (data not shown).

The concentration of total phenolics increased until day 3 and was consistently lower in the ethanol treated tissues (Fig. 10D). However, phenolic concentration changes were not related with changes in respiration or PAL activity. Results reported here with ethanol treated sunchoke agree with research on fresh-cut potatoes by Cantos et al. (2002) and Tsouvaltzis et al. (2011). With fresh-cut lettuce, PPO activity was associated with increased phenolics concentration and browning in some studies (Luna et al., 2012, 2013), but others found PAL activity more closely associated with discoloration (Hisaminato et al., 2001; Loaiza-Velarde and Saltveit, 2001; Loaiza-Velarde et al., 2003). Mishra et al. (2013) reported that browning in eggplant tissue was dependent on soluble phenolics and PPO activity. These differences suggest variation in the mechanism or regulation of discoloration in different commodities.

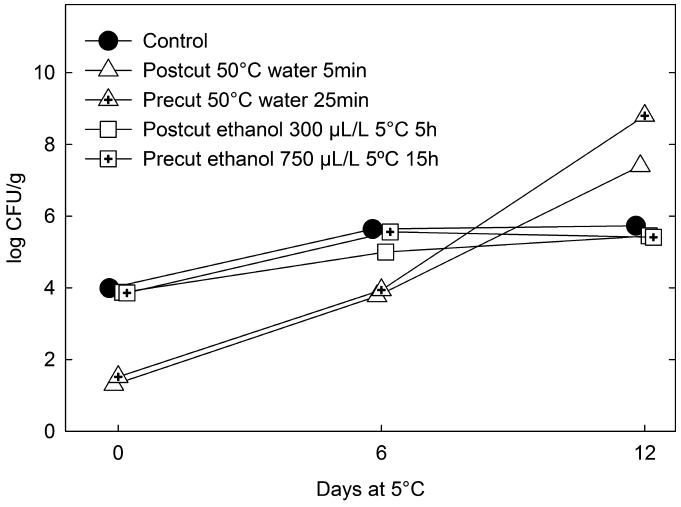

Fig. 11. Total aerobic plate counts (log cfu/g) of sunchoke slices stored for 0, 6 and 12 d at 5 °C. Slices were untreated or received pre-cut (50 °C 25 min) or post-cut (50 °C 8 min) hot water dips, or pre-cut (750 µL/L 15 h) or post-cut (300 µL/L 5 h) ethanol fumigation. Data are the means of duplicates.

3.5. Hot water and ethanol treatments and microbial growth

On day 0, the total plate counts of pre- and post-cut hot water treated sunchoke slices were significantly lower than those of control slices (Fig. 11), and the lower count was maintained until day 6. However, the heat treatments had a negative effect from day 6 to day 12. These treatments had previously shown to sometimes cause visible tissue damage on the slices by day 12 (Fig. 3) and in the microbiology test, slices treated with hot water began to soften gradually from day 8 (data not shown).

The two ethanol treatments had no effect on total microbial load over the 12 day period (Fig. 11). As with the untreated slices, there was not a significant increase in aerobic plate counts over the 12 d period. It has been shown however, that at doses of 1.1–1.8 g/kg, ethanol could reduce microbial loads on fresh-cut mango (Plotto et al., 2006).

4. Conclusions

Hot water or ethanol treatments can effectively inhibit discoloration of fresh-cut sunchoke tubers. Sunchoke slices post-cut treated with water at 50 °C water for 6–8 min, 55 °C for 3–4 min and tubers pre-cut treated with 50 °C water for 20–25 min maintained good visual quality with little discoloration for 8–12 d at 5 °C. Post-cut ethanol fumigation (300–750 µL/L for 5 h at 5 °C) inhibited discoloration for more than 30 d at 5 °C. Post-cut dipping with ethanol solutions (5, 8 or 10%) extended shelf-life up to 15 d. Ethanol treatments inhibited undesirable discoloration, but also substantially reduced respiration rates and PAL activity. Post-cut 10% ethanol dips also inhibited wound-induced respiration, PAL activity and total phenolic concentration. No clear relationship was found between PPO activity, total phenolic concentration

and discoloration scores in sunchoke slices. Both the hot water and ethanol treatments could be effective, but ethanol dip treatments were the easier to apply with a broad range of effective concentrations and little risk of product injury.

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