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The mechanism of ethanol treatment on inhibiting lettuce enzymatic browning and microbial growth

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ABSTRACT

Tissue browning greatly affects the quality and consumer acceptance of fresh-cut lettuce. Unfortunately, effective antibrowning agents that either have antimicrobial activity or compatibility are hard to find. This study investigated the effects of ethanol treatment on enzymatic browning and microbial growth on lettuce stem discs. Lettuce stem discs were treated with ethanol (200 mL/L) for 2 min, drained by salad spinner, packaged in Ziploc® bags and stored at 5 °C. Stem discs treated with ethanol exhibited better appearance and lower microbial loads than untreated samples within 14 days of storage time. Phenylalanine ammonia lyase mRNA level and enzymatic activities in the ethanol treated group were significantly suppressed, and total phenols and quinones were also reduced. In addition, ethanol treatment increased CO_2 production and reduced O_2 level inside the package. In comparison to heat shock, trans-cinnamaldehyde and salicylic acid immersion, ethanol treated lettuce stem discs had the least browning and best quality attributes. These results indicated that 200 mL/L ethanol treatment was the most effective approach to inhibit enzymatic browning and control microbial growth on lettuce stem discs.

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1. Introduction

Enzymatic browning of fresh-cut fruits and vegetables is a costly problem for the fresh-cut produce industry because it has an adverse effect on appearance, aroma, flavor, and nutritional value (Toivonen & Brummell, 2008), detracts from customer acceptance, and reduces product shelf life. The main enzyme responsible for browning in fruits and vegetables is polyphenol oxidase (PPO), which catalyzes the oxidation of polyphenols to form colored quinones. In intact tissues, the enzyme is located in the cytoplasm while their substrates are located in the vacuoles (Chazarra, Garcia-Carmona, & Cabanes, 1999). Damaging lettuce tissue by cutting results in the mixing of the enzyme and substrates, and exposures to oxygen leading to the browning reaction (Degl'Innocenti, Guidi, Pardossi, & Tognoni, 2005). Saltveit (2000) suggested that altered phenol metabolism was involved in browning of lettuce leaf tissue. Polyphenols are produced by a complex set of interrelated metabolic reactions: 1) the shikimate pathway by which phenylalanine is produced from phosphoenolpyruvate and erythrose 4-phosphate; 2) the phenylpropanoid pathway by which cinnamic acid, a precursor for the biosynthesis of polyphenols are generated; and 3) browning reactions by which polyphenols are oxidized to form quinones. Phenylalanine ammonia-lyase (PAL), which catalyzes the conversion of L-phenylalanine to cinnamic acid, is the rate-limiting enzyme in this series of reactions. Cutting or wounding can induce the PAL expression and increase its activity which leads to the accelerated tissue browning (Hisaminato, Murata, & Homma, 2001; Llorach, Martínez-Sánchez, Tomasbarberan, Gil, & Ferrers, 2008).

Control of enzymatic browning has always been a challenge to the food industry (Altunkaya & Gökmen, 2009). Sulfites have long been used as both antimicrobial preservatives and antibrowning agents, because they alter products generated in enzymatic reactions by forming colorless adducts and inactivate PPO by binding to the enzyme irreversibly. However, sulfites alter flavor and aroma, destroy thiamine and possibly other dietary components, and are currently restricted due to the hazard of allergic-like reactions in sulfite-sensitive asthmatics (U.S. Food and Drug Administration, 1986, 1990). The reducing agents, ascorbic acid and L-cysteine were found to be competitive inhibitors of PPO in lettuce

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(Altunkaya & Gökmen, 2008). The inhibitory effect of ascorbic acid was temporary and declined as ascorbic acid was converted to dehydroascorbic acid (Altunkaya & Gökmen, 2008). Cysteine was found to be a more effective inhibitor of PPO (Altunkaya & Gökmen, 2008). Cysteine forms colorless cysteine-quinone adducts during enzymatic oxidation which are competitive inhibitors of PPO (Richard-Forget, Goupy, & Nicolas, 1992). Acidulants such as citric acid have also been used extensively as PPO inhibitors. Citric acid exerts a double inhibitory effect on PPO, by lowering the pH (below that necessary for optimal PPO activity) and by chelating copper (Ibrahim, Osman, Sasari, & Abdul Rahman, 2004). Sodium chlorite was demonstrated to inhibit PPO and inactivate *E. coli* O15:H7 on fresh-cut apples (Luo, Lu, Zhou, & Feng, 2011). However, it caused too extensive tissue damage in fresh-cut lettuce at concentrations that were effective to prevent browning (Luo, unpublished).

It has been shown that there is a correlation between PAL activity and browning in lettuce leaves (Hisaminato et al., 2001; Murata, Tanaka, Minoura, & Homma, 2004; Tanaka et al., 2011). CO_2 can inhibit browning of physically damaged plant tissue by lowering the level of phenolic compounds (Siriphanich & Kader, 1985). Several other physical and chemical methods can control browning by affecting PAL. Heat shock (HS) or mild heat treatment at 50–60 °C inhibited enzymatic browning of cut lettuce by suppressing cutting-induced PAL activity (Murata et al., 2004). Salicylic acid (SA) (Peng & Jiang, 2006) and trans-cinnamaldehyde (Martiñon, Moreira, Castell-Perez, & Gomes, 2014) in appropriate concentration were also used to control browning in fruits and vegetables. However, there is no antibrowning agent with merit of effectively controlling microbial growth of spoilage and pathogen in fresh-cut produces. Ethanol is a generally recognized as a safe (GRAS) product and exits naturally in plants with low toxicity for fruits and vegetables. It has been used to retard tissue senescence (Perata & Alpi, 1991), maintain the quality of intact apples, grape, cherry, peaches, mango, fresh-cut eggplant (Chervin, Westercamp, & Monteils, 2005; Hu, Jiang, Tian, Liu, & Wang, 2010) and asparagus spears (Herppich, Huyskens-Keil, & Hassenberg, 2014). In this work we evaluated the effect of ethanol application to simultaneously control the browning and microbial growth of lettuce tissue after it was wounded by cutting. We also compared the antibrowning effects of ethanol treatment with other recently reported inhibitors.

2. Materials and methods

2.1. Lettuce stem discs and storage

Commercially grown and harvested crisp-head iceberg lettuce (*Lactuca sativa* L.) were purchased from a local retailer and stored at 1.5 °C. About 3000 stems were cored from the lettuce head. After removing the top and bottom portion of each stem, the middle portion was cut into discs with 2 mm thickness (about 2 g) each according to Tomás-Barberán, Gil, Castañer, Artés, and Saltveit (1997). About 5–7 discs were obtained from each stem.

2.2. Treatment of stem discs

Twenty five lettuce stem discs (about 50 g) for each treatment were immersed into 0, 100, 200 and 300 mL/L (v/v) ethanol solution (diluted with distilled water) at 15 °C for 2 min. Excess moisture was removed using a salad spinner. Untreated stem discs served as control. Treated and untreated discs were sealed in Ziploc® sealable polyethylene bags (18 cm × 16 cm) and stored at 5 °C for 21 days. Similarly, the stem discs were treated with 0.5 g/L *trans*-cinnamaldehyde solution (CD) at 15 °C for 30 min (Martiñon et al., 2014), heatshock (HS) by immersing in 50 °C water for 1.5 min (Murata et al., 2004), or 1 g/L salicylic acid solution (SA) at 15 °C for 1 min (Peng & Jiang, 2006).

2.3. Reagents

Ethanol was purchased from PHARMCO-AAPER (CT, USA), trans-cinnamic acid was purchased from Aldrich Chemical Company, Inc. (WI, USA), *trans*-cinnamaldehyde, phenylalanine and salicylic acid were purchased from Sigma–Aldrich, Inc. (MO, USA).

2.4. Evaluation of tissue browning

A Minolta Chroma Meter CR-300 (Minolta Corp., Osaka, Japan) was used to assess the color of lettuce stems discs. In order to account for within-stem and within-bag color variation among cut surfaces of lettuce stem discs, two measurements per lettuce stem disc and six measurements per bag were taken. The means of L^* a^*, and b^* from 12 readings were recorded for each replication on each sampling day. The Chroma meter was calibrated with a standard white plate ($Y = 94.00$, $x = 0.3158$, $y = 0.3322$). Color was measured on day 0, 4, 7, 14 and 20.

2.5. Enzyme extraction and assay

PAL activity was measured as described (Degl'Innocenti et al., 2005) with some modification. About two g frozen lettuce stem discs from the mixture of 25 discs were homogenized at 4 °C with 16 mL of 50 mmol L^{-1} borate buffer (pH 8.5) containing 5 mmol L^{-1} 2-mercaptoethanol and 0.2 g of polyvinylpyrrolidone. The homogenate was filtrated through 4 layers of cheesecloth and centrifuged at 20,000 × *g* at 4 °C for 10 min. The supernatant was assayed for PAL activity after the addition of 0.55 mL of 50 m mol L^{-1} L-phenylalanine and incubation at 40 °C for 1 h. The samples were measured at 290 nm using the Shimadzu PharmaSpec UV-160A UV–Vis spectrophotometer (Shimadzu Scientific Instrument, MD, USA) before and after incubation. One unit of PAL activity equals the amount of PAL that produced 10^{-3} mol of transcinnamic acid per kg fresh discs in 1 h. PAL activity was measured daily from day 0 to day 6 of storage.

2.6. Polyphenol compound determination

Phenolic compounds were extracted as previously described (Ke & Saltveit, 1988). Briefly, 2.5 g of tissue was homogenized with 5 mL of methanol using a VWR VDI 25 homogenizer (VWR International LLC., PA, USA) at high speed for 30 s. The homogenate was filtered through four layers of cheesecloth and centrifuged at 15,000 × *g* for 15 min. The supernatant was used directly to measure the browning potential and soluble o-quinones. The absorbance of an aliquot of the supernatant was measured at 320 nm to determine the browning potential, and at 437 nm to determine the relative concentration of soluble o-quinones using the Shimadzu UV-160A UV-VIS recording spectrophotometer. Measurements were taken daily from day 0 to day 6 of storage.

2.7. Analysis of microbiological profile

Lettuce stem discs (10 g) from each package were macerated in 90 mL sterile phosphate buffered saline (PBS) for 25 min and then homogenized for 1 min. The lettuce stem solution was then serially diluted with PBS, and plated on an appropriate medium using spiral plater (Microbiology International, MD, USA). The aerobic mesophilic bacterial count (AMB) and yeast and mold (YM) counts were determined by plating the samples on trypticase soy agar (BD, NJ, USA) incubating at 36 °C for 24 h, and on potato dextrose agar (BD,

NJ, USA) incubating at 28 °C for 72 h, respectively. Microbial assays were conducted on day 0, 3, 7, 14, and 21.

2.8. Analysis of package headspace atmospheric composition

The partial pressures of O_2 and CO_2 in the packages were determined using a Checkmate 9900 analyzer (PBI Dansensor, Ringsted, Denmark). Without opening each bag, a gas sample was taken by inserting a needle with a diameter of 0.4635 mm connected to the measuring apparatus through a rubber septum adhered to the package. Gas measurements were taken 2 h after processing on day 0, 1, 2, 3, 5, 7, 9, and 14.

2.9. Real-time quantitative reverse transcription PCR

Frozen lettuce stem discs (about 0.2 g) were pulverized in liquid nitrogen. Total RNA was extracted and purified with plant RNA Extraction Kit (Invitrogen, CA, USA) according to the manufacturer's instructions. Total RNA (300 ng) was reverse-transcribed to cDNA using iScript Reverse Transcription Supermix (Bio-Rad, CA, USA). Gene-specific primers were designed according to two Romaine lettuce *PAL* genes in Genbank (*PAL-1*, AF299330; and *PAL-2*, AF411134). Ubiquitin was used as an internal control. The forward and reverse primer sequences were as follows: *PAL1*, TTAGTCGCTCTATGCCAATCCA/AGCGAAGACGTATTCACGATCA; *PAL2*, CCCACCGGAGAAGTTCTAAATG/ACTTCCGACAACAATGCAAGAA, and Ubiquitin (Ubi), AAGACCTACACCAAGCCCAA-3/AAGTGAGCCCCA-CACTTACCA-3′. qPCR was performed with a GoTaq qPCR master Mix (Promega, WI, USA). PCR conditions were set as follows: 95 °C for 4 s, 60 °C for 25 s and 72 °C for 5 s by 45 cycles.

2.10. Statistical analysis

Experiments were conducted with three replications per treatment. Data were analyzed for statistical significance using the OriginPro 8 (OriginLab, MA, USA), and presented as means with standard deviations. Mean values of three replicates were compared using the LSD (least significant difference) test after two-way analysis of variance (ANOVA) with storage time and treatments as variability factors.

3. Results and discussion

3.1. Ethanol treatment retarded enzymatic browning

Lettuce stem discs were treated with different concentrations of ethanol. Among these treatment, 200 mL/L ethanol exhibited the best effect by maintaining pale green color during the storage period. However, untreated control browned significantly as evidenced by the lower lightness, L* value, and higher in redness a* and yellowness b* in the surface of fresh-cut lettuce stem discs stored at 5 °C (Fig. 1A, B, C). Notably that the L* values decreased rapidly in control but remained relatively stable in ethanol treated samples during 14 days of storage. The a* values in the control group increased rapidly at the beginning of the storage and reached a plateau on day 14, while those of the ethanol treatment group remained constantly low. The a* values for untreated control were significantly higher ($p < 0.05$) than those for ethanol treated samples on all evaluation days after day 0. The b* color values for both ethanol-treated stem discs and controls increased during storage. However, the b* values for treated samples did not increase as rapidly or to as great extent as those for controls, indicating that the ethanol treatment retarded the yellowing of the lettuce stem discs. As shown in Fig. 1D, pink and brown dots appeared on the cut surface of controls at day 5. However, the cut surface of ethanol treated discs had only very faint browning around the outer edge. These results show that the 200 mL/L ethanol treatment significantly inhibited the browning of lettuce stem discs.

3.2. Ethanol treatment affected phenylalanine ammonia-lyase gene expression and activity

There are two *PAL* genes in lettuce. After cutting, expression of both *PAL1* and *PAL2* genes were rapidly increased in controls within 2 h, reached a peak at 6 h, and then decreased quickly to the basal level by 50 h (Fig. 2). However, the expression levels of the two *PALs* in ethanol treated discs were significantly reduced as compared to controls. In particular, *PAL2* expression was hardly detected in ethanol treated discs. Although we detected an expression peak of *PAL1* at 12 h after ethanol treatment, its expression level was only about half of that observed in the control group. These results indicated that ethanol immersion can quickly repress the expression of both *PALs* in lettuce stem after cutting.

Further we investigated whether the PAL activity was affected by ethanol treatment. The PAL activity in the control group after cutting increased very rapidly, peaked within two days and then decreased (Fig. 3). However, in treated samples the PAL activity rose gradually until the 3rd day and then leveled off at around 0.6 mol kg^{-1} FW h^{-1}. The largest difference occurred on the 2nd day, when the PAL activity in the ethanol treated samples was only about 10% of the untreated control. After the 3rd day, the PAL activity in the treated samples and untreated controls became closer, although it remained higher in the control. Altogether these results indicated that cutting/wounding stimulated *PAL* gene expression and activity. However, the ethanol treatment can effectively repress the *PAL* gene expression and reduce PAL activity.

3.3. Ethanol treatment altered the phenol and quinone contents

The phenolic compounds synthesized by PAL can be oxidized, by PPO to quinones, which spontaneously polymerize to brown pigments responsible for tissue browning (Ke & Saltveit, 1986, 1989). It has been reported that ethanol retardation of browning development resulted from its inhibiting effects on the formation of reactive oxygen species and the activity of various oxidases (Hu et al., 2010). Thus we compared the changes in contents of total phenolic compounds and quinones in different treatments during 6 days storage. Soluble phenolic compounds accumulated in the control group after cutting, reached a peak on day 4, and then decreased. The soluble phenolic compounds in the ethanol treated discs also increased during storage, but lagged behind the controls. However, they surpassed them in the control group on day 5 and peaking on day 6 (Fig. 4A). Choi, Tomás-Barberán, and Saltveit (2005) reported that treatment with 0.1 mol L^{-1} 1-butanol for 2 h or exposure to 0.02 mol kg^{-1} FW 1-butanol vapors for 12 h reduced the accumulation of wound-induced phenolic compounds in lettuce mid-rib tissue. Quinone concentration in all samples increased after cutting, fluctuated, and reached a peak at day 4 and then decreased. However, in ethanol treated samples, quinone concentration remained lower and kept more stable than the control (Fig. 4B). These results suggest that ethanol treatment can reduce the accumulation of polyphenols and quinones so as to retard the browning.

3.4. Ethanol treatment affected the O_2 and CO_2 concentrations within the packages

Mateos, Ke, Kader, and Cantwell (1993) reported that exposure to carbon dioxide concentration above 2% may cause a disorder named “brown stain” in lettuce. Unlike whole heads, minimally

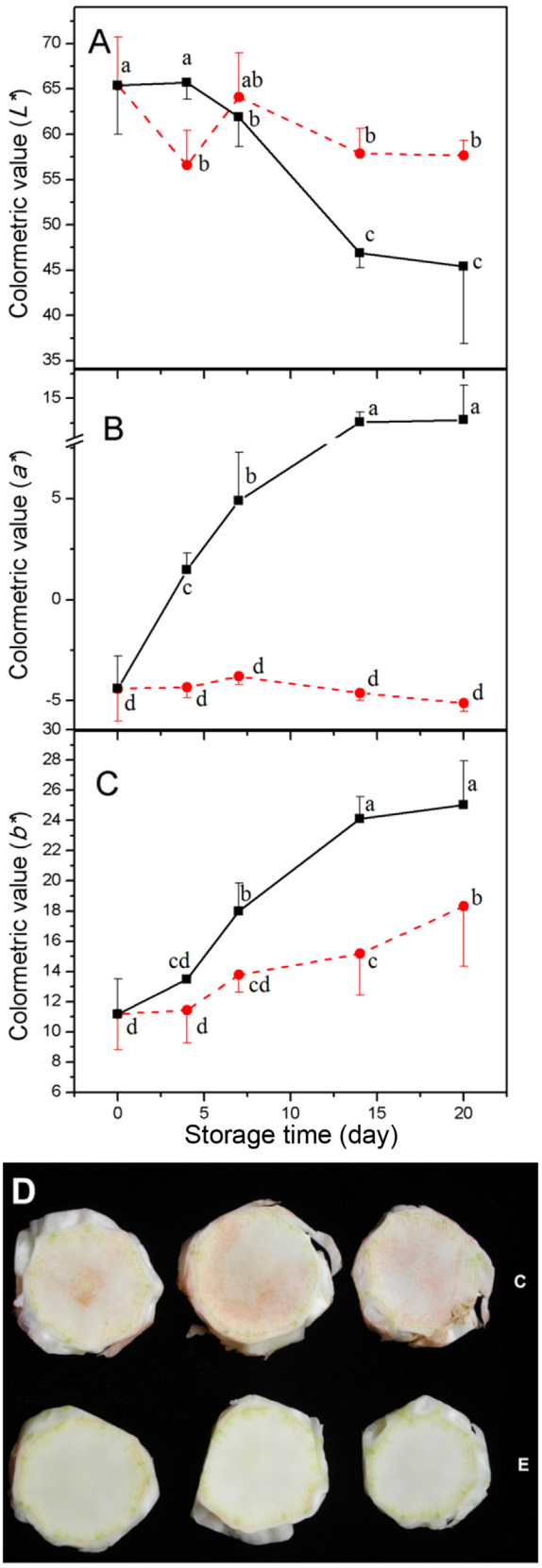

Fig. 1. Effect of ethanol treatment on the color of lettuce stem discs during storage at 5 °C. Color parameters: (A) L* (from 0 black to 100 white); (B) a* (from −a* green to +a* red) and (C) b* (from −b* blue to +b* yellow). Data presented are the means of six replicates. Different letters indicate significant differences among mean values ($P < 0.05$; *t*-test). ■C: untreated control; ●E: 200 mL/L ethanol treatment. (D) Antibrowning effect of ethanol on lettuce stem discs. The photographs were taken on day 5 after treatment. Upper panel, untreated controls; Lower panel, ethanol treated stem discs.

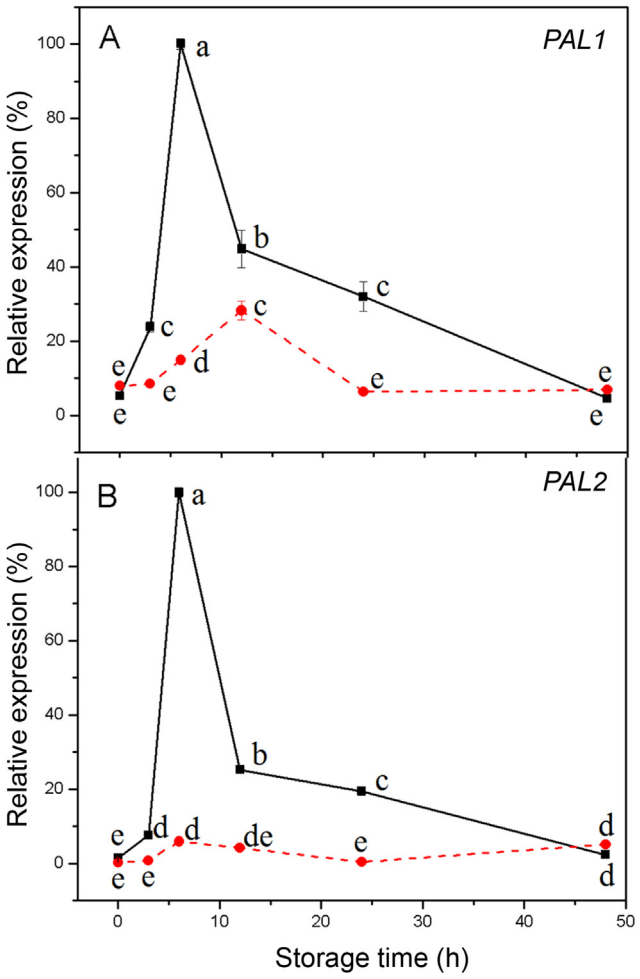

Fig. 2. Effect of 20 mL/100 mL ethanol on the mRNA levels of phenylalanine ammonia lyase *PAL1* (A) and *PAL2* (B) in cut lettuce stem discs during cold storage. Transcription levels of *PALs* genes were analyzed by RT-qPCR. Relative gene expression levels (highest value = 100%) were shown following normalization with *Ubiquitin* transcript values. Data presented are the means of three replicates. Different letters indicate significant differences among mean values ($P < 0.05$; *t*-test). ■C: untreated control; ●E: 200 mL/L ethanol treatment.

processed lettuce was less sensitive to carbon dioxide injury (McDonald, Risse, & Barmore, 1990). In this study, the ethanol treatment stimulated CO_2 production and inhibited O_2 concentration by the stem discs as compared to those in the control group (Fig. 5). The oxygen concentration in the control packages decreased from 21 kPa to the lowest point 17.5 kPa on the first day of the storage period, then increased gradually and got close to the initial value by the end of the storage. However, O_2 concentration in the ethanol treated packages decreased from 21 kPa to the lowest point 12.5 kPa on the second day and then rose gradually to 15 kPa. The carbon dioxide concentration in the ethanol treatment and the control packages followed the complementary pattern, i.e. quickly increased in the first day after cutting, and then decreased. Nonetheless, the ethanol treated discs released more CO_2 than untreated discs during the entire storage period. For example, in ethanol treated discs, CO_2 reached 3 kPa on the first day and then decreased gradually to around 2.3 kPa by day 14. In contrast, CO_2 in control

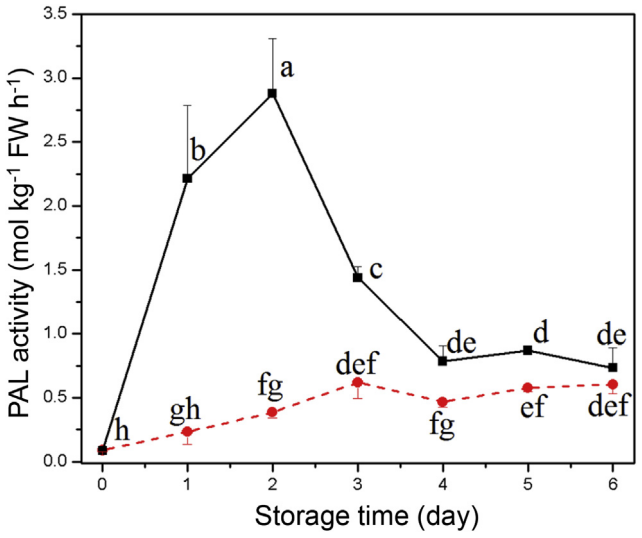

Fig. 3. Changes of phenylalanine ammonia lyase (PAL) activity in ethanol treated lettuce stem discs during storage. Data presented are the means of six replicates. Different letters indicate significant differences among mean values ($P < 0.05$; *t*-test). ■C: untreated control; ●E: 200 mL/L ethanol treatment.

packages climbed only to 2 kPa on the first day, and then gradually decreased to 0.5 kPa. Ethanol has been shown to have a slight stimulatory effect on the respiration in asparagus spears (Herppich et al., 2014). However, ethanol treatment did not influence the respiration in broccoli florets (Corcuff, Arul, Hamza, Castaigne, & Makhlouf, 1996), and actually repressed respiration in mango fruit (Plotto, Bai, Narciso, Brecht, & Baldwin, 2006). Our results indicate that ethanol significantly increased the ratio of CO_2/O_2 inside the packages. Thus it will be very interesting to study the underlying mechanisms. Low oxygen atmospheres inhibit browning since oxygen is required for the oxidation of phenols to quinones. CO_2 is known to inhibit browning in lettuce by lowering the level of phenolic compounds (Siriphanich & Kader, 1985). Thus, ethanol may inhibit browning by several mechanisms including the direct effect on repressing PAL, as well as the indirect effects on depressing O_2 and elevating CO_2 levels.

3.5. *Effect of the ethanol treatment on the microbial growth*

Most browning inhibitors cannot reduce microbial growth even early in the shelf-life and may actually stimulate it (Moreira, Ponce,

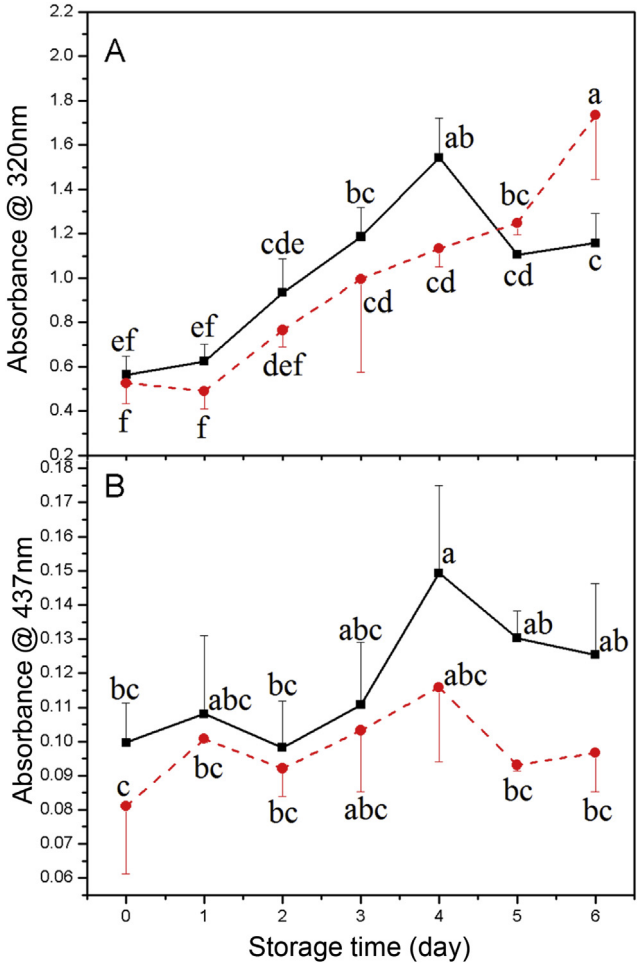

Fig. 4. Total phenolic changes in untreated control (■ C) and ethanol treated (●E) lettuce stem discs on day 1–6. (A) Total phenols in absorbance at 320 nm; (B) Total quinones in absorbance at 437 nm. Data presented are the means of six replicates. Different letters indicate significant differences among mean values ($P < 0.05$; *t*-test).

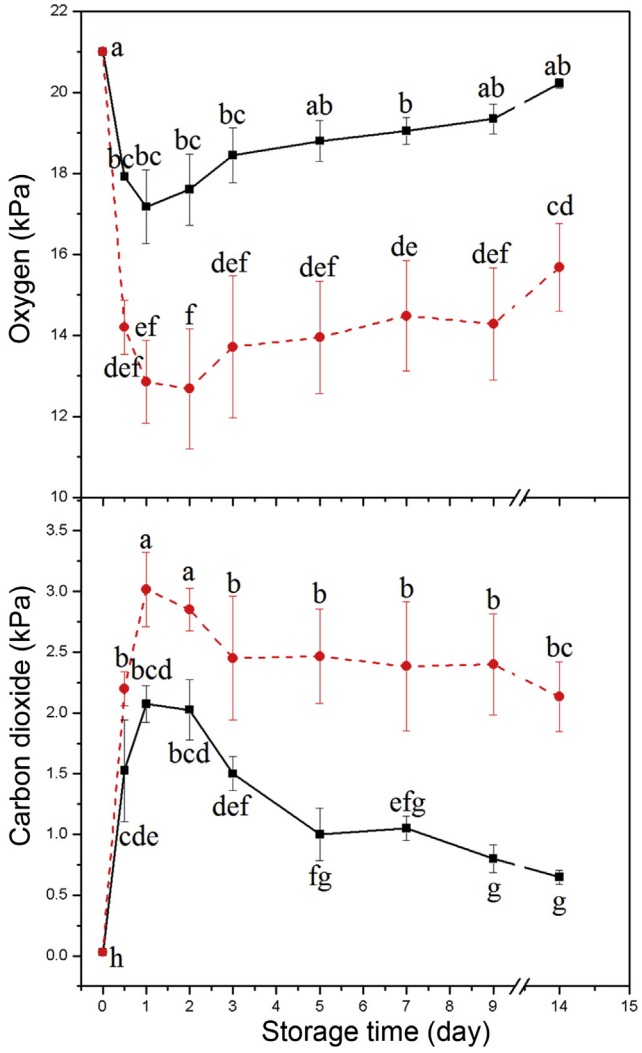

Fig. 5. Changes in gas composition inside the packages of untreated control (■C) and ethanol treated (●E) lettuce stem discs during storage. Data presented are the means of six replicates. Different letters indicate significant differences among mean values ($P < 0.05$; *t*-test).

Del Valle, & Roura, 2004; Murata et al., 2004; Tanaka et al., 2011). For example, heat shock treatment has been reported to increase microbes on treated vegetables during storage (Moreira et al., 2004; Murata et al., 2004). The number of natural microbes and surviving pathogens observed on inoculated fresh-cut lettuce treated with cinnamaldehyde was similar to that of non-treatment control after 5–12 days storage at 4 °C (Tanaka et al., 2011). Our study showed that the bacterial, and yeast and mold counts on ethanol treated discs were significantly lower than those on controls for the first 14 days of storage (Fig. 6). On day 14, although the microbial counts on ethanol treated cut lettuce were still lower than those of the controls, the difference was less pronounced. By day 21 the microbial counts in the ethanol treated lettuce surpassed those in the controls. This could be resulted from some tissue damage by the ethanol treatment which led to increased microbial growth after 14 days. Herppich et al. (2014) also reported that ethanol at low concentration can reduce microbial growth on fresh white asparagus spears, especially during prolonged storage of 4 days. Our results suggest that ethanol treatment had dual benefits, antibrowning and antimicrobial for the fresh-cut lettuce within 14 days of storage.

3.6. Comparison of ethanol treatment with other methods on antibrowning effect

We further compared the antibrowning effect of the ethanol treatment to three previously reported methods: heat shock (hot water), *trans*-cinnamaldehyde and salicylic acid solution immersions. All treatments were able to inhibit enzymatic browning based on the a* value for the first 7 days of storage (Fig. 7). From day 7 to day 14, the heat shock treatment was equally as effective as the 200 mL/L ethanol solution, while the other treatments had less antibrowning effect (*trans*-cinnamaldehyde) or even stimulated browning (salicylic acid) (Fig. 8). Even though there was not a significant difference on the overall color, the cut surface of lettuce stem discs treated with heat shock displayed a pink ring of dots on day 14. Thus ethanol treatment was the most effective for maintaining quality of lettuce stem discs within 14 day storage period.

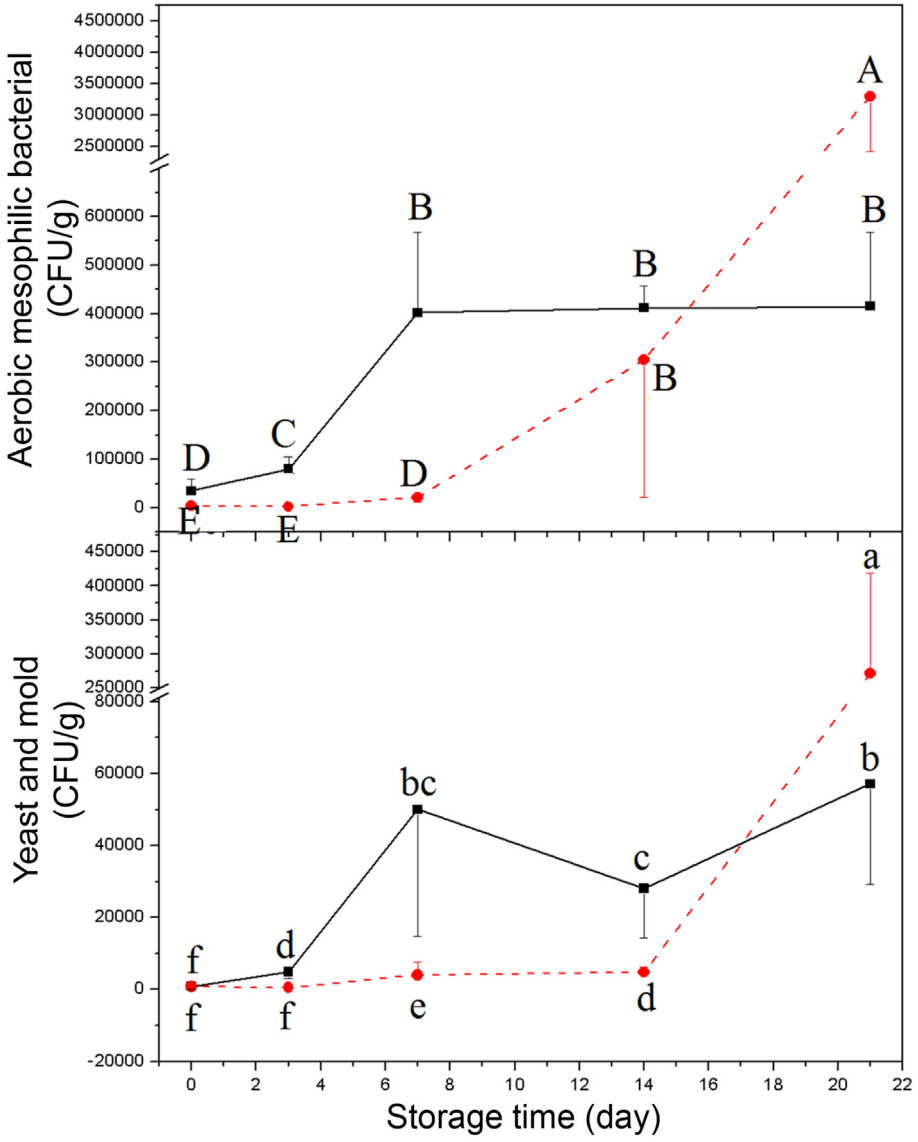

Fig. 6. Effect of ethanol treatment on the natural microflora growth during cold storage for untreated control (■C) and ethanol treated (●E) lettuce stem discs. Data presented are the means of six replicates. Different capital and small letters indicate significant differences, $P < 0.01$ and $P < 0.5$, among mean values, respectively.

4. Conclusions

The quality of fresh-cut lettuce is diminished by wound-induced browning. Increased demand for minimally processed lettuce and tighter restriction on chemical treatment demands improved understanding of enzymatic browning, as well as simple and natural treatments to control it (Martin-Diana et al. 2005). Accumulated evidence indicates that browning in fresh-cut lettuce is caused by the increased PAL activity and accumulation of phenolic compounds stimulated by tissue damage. Treatment of lettuce stem discs with 200 mL/L ethanol significantly retarded browning. Ethanol treatment drastically repressed the expression of *PAL* mRNA, inhibited PAL activity, and increased the ratio of CO_2/O_2. Therefore ethanol treatment was able to inhibit phenolic metabolism which is critical for tissue browning. Moreover, ethanol treatment was more effective than other antibrowning methods, such as cinnamaldehyde, heatshock and salicylic acid, for maintaining quality of lettuce stem discs. In short, ethanol was demonstrated to be effective for the dual functions of inhibiting browning and microbial growth during 14 days of storage and therefore has great potential for use as a commercial treatment for fresh-cut lettuce.

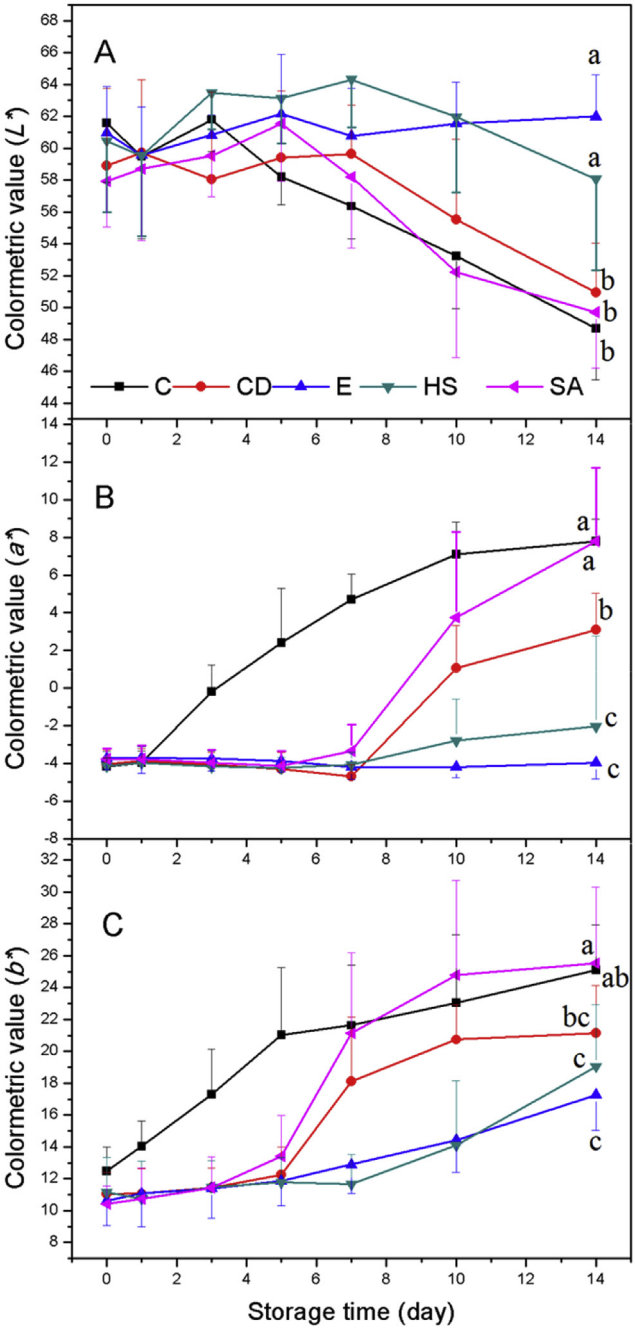

Fig. 7. The effect of different treatments on color changes during storage detected by colorimeter. (A) L* (from 0 black to 100 white); (B) a* (from −a* green to +a* red); (C) b* (from −b* blue to +b* yellow). C: untreated, CD: treated with 0.5 g/L *trans*-cinnamaldehyde for 30 min, E: treated with 200 mL/L ethanol water solution for 2 min; HS: treated in 50 °C water for 90 s; SA: treated with 1 g/L salicylic acid for 1 min. All treatments were applied immediately after cutting. Data presented are the means of six replicates. Different letters indicate significant differences among mean values ($P < 0.05$; *t*-test).

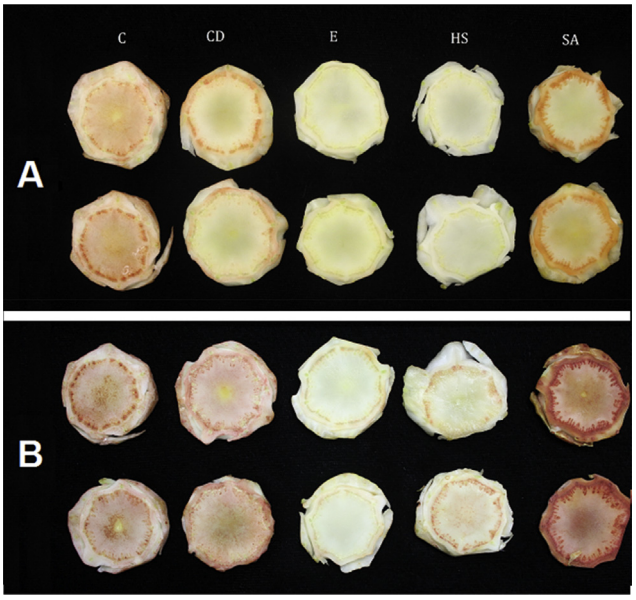

Fig. 8. Photographs showing the antibrowning effects of different treatments on lettuce stem discs on day 7 (A) and day 14 (B). C: untreated control; CD: treated with 0.5 g/L *trans*-cinnamaldehyde for 30 min; E: treated with 200 mL/L ethanol for 2 min; HS: treated in 50 °C water for 90 s; SA treated with 1 g/L salicylic acid for 1 min. All treatments were applied immediately after cutting.

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