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Original article

# Reassessment of treatments to retard browning of fresh-cut Russet potato with emphasis on controlled atmospheres and low concentrations of bisulphite

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**Summary** The cultivar Pacific Russet with high browning susceptibility was used for most testing. Controlled atmospheres (0.3%, 3% and 21% $O_2$ in combination with 0%, 6% or 12% $CO_2$) and anti-browning chemicals were studied in relation to quality retention and wound-induced phenolic metabolism of fresh-cut slices for up to 16 days at 5 °C. The 3% $O_2$ + 12% $CO_2$ atmosphere was most effective among those tested, and retarded increases in phenolics and phenylalanine ammonia lyase activity, but had only slight benefit on visual quality. A 1.25% ascorbic acid + 1.25% citric acid treatment was ineffective, but when combined with 3% $O_2$ + 12% $CO_2$, it was comparable with 0.025% sodium bisulphite. Bisulphite concentrations from 0.05% to 0.25% provided similar effective control of discolouration. Bisulphite as low as 0.025% with 3% $O_2$ + 12% $CO_2$ resulted in a visual quality score at the limit of marketability after 8 days at 5 °C. Chemical treatments did not retard increases in phenolic concentrations or phenolic enzyme activities.

**Keywords** Ascorbic acid, citric acid, controlled atmosphere, colour values, phenylalanine ammonia lyase, polyphenol oxidase, phenolics, sodium bisulphite, *Solanum tuberosum*.

## Introduction

Consumer demand for fresh, nutritious, and convenient fruits and vegetables has spurred the growth of fresh-cut products. Fresh-cut preparation triggers many physiological and biochemical changes, such as increased respiration rates and ethylene production rates, induced synthesis of secondary metabolites, membrane disruption and activation of defence mechanisms (Brecht *et al*., 2004).

The enzymatic browning of cut surfaces leads to loss of marketable quality and remains a major challenge for many fresh-cut products (Laurila *et al*., 1998a; Toivonen & Brummell, 2008). Retardation of undesirable browning typically involves control of phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO) and other enzyme activities, concentrations of phenolic compounds, pH, temperature, oxygen availability and other factors (Saltveit, 1997). Potatoes have relatively high concentrations of preformed phenolic compounds and therefore control of PPO activity and polymerisation reactions are required to minimise undesirable browning reactions in the fresh-cut products (Friedman, 1997).

Ascorbic acid (AA) can inhibit enzymatic browning, primarily due to the reduction of quinones before they undergo condensation to form coloured pigments (Martinez & Whitaker, 1995). AA may also chelate the copper at the active site of PPO. However, once AA has been completely oxidised to dehydroascorbic acid, quinones accumulate and undergo polymerisation reactions leading to browning (Friedman, 1997). Citric acid (CIA), another widely used anti-browning agent, has a dual inhibitory effect by lowering the pH and chelating the copper at the active site of PPO (Martinez & Whitaker, 1995). AA is often applied in combination with CIA for improved efficacy (Laurila *et al*., 1998b; Limbo & Piergiovanni, 2006, 2007; Rocculi *et al*., 2007).

Chemicals containing SH-groups including sulphites are employed to prevent browning in vegetables such as potatoes (Laurila *et al*., 1998b). Sulphites retard microbial activity and inhibit enzymatic (competitive inhibitor of PPO) and nonenzymatic browning reactions (Beltrán *et al*., 2005). Sulphites may also form intermediate sulphoquinones that inhibit PPO activity (Marshall *et al*., 2000). Although sulphites can cause allergic reactions in some people, they are allowed at low

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concentrations in potato products as most of the sulphite is lost during cooking (FDA, 2010; FMI, 2010). Because sulphites are low cost and very effective, it has been a challenge to find efficacious anti-browning replacements (Sapers *et al*., 1990; Gunes & Lee, 1997; Ahvenainen *et al*., 1998; Laurila *et al*., 1998b).

Modified or controlled atmospheres (MA or CA) with low oxygen and/or high carbon dioxide concentrations are used for potatoes and other fresh-cut products to retard browning reactions (Gunes & Lee, 1997; Beltrán *et al*., 2005; Angós *et al*., 2007; He & Luo, 2007). As browning involves oxidation, it can be retarded by eliminating oxygen at the cut surface. However, lack of oxygen leads to anaerobiosis with an undesirable increase in fermentative volatiles, and once the package is open, rapid browning can occur when oxygen is reintroduced (Mateos *et al*., 1993). Although useful concentrations vary by product, atmospheres of 1–3% $O_2$ and 8–10% $CO_2$ have been found effective for many cut vegetables (Brecht *et al*., 2004). MA in combination with anti-browning chemicals are often more effective than either used alone (Brecht *et al*., 2004; Limbo & Piergiovanni, 2007).

Fresh-cut processors may not have control over the variety used and therefore may need to prepare potatoes with differing tendency to browning. One objective of this study was to re-evaluate various proposed anti-browning treatments using a single source of potato with high browning potential. A second objective was to study very low concentrations of sulphites, with and without controlled atmospheres, to determine the concentration limit for efficacy. Evaluations included visual quality and discolouration, objective colour values, analyses of phenolic enzymes, and total and individual phenolics.

## Materials and methods

### Plant material and treatments

Several cultivars along with a large quantity of cv Russet Pacific potatoes were purchased from a local wholesaler and stored at 7.5 °C until used. Potatoes for the control treatment were scrubbed-washed, rinsed in chlorinated water (200 ppm NaOCl pH 7.0), cut at 7.5 °C into 5 mm (±0.5 mm) slices manually with a stainless steel mandoline, rinsed in 50 ppm NaOCl (pH 7.0), and patted with paper towels. For chemical treatments, slices were prepared as in the control treatment and then dipped in freshly prepared solutions (no pH adjustment; volume: weight ratio of 10:1) for 3 min at 7.5 °C and patted dry. Slices were placed in vented polyethylene bags which were stored in polycarbonate plastic containers with a flow of humidified air or controlled atmosphere, the latter kept within 5% of the stated concentrations. Slices were evaluated after 0, 4, 8, 12 and 16 days.

### Colour

Surface colour of the slices was determined with a Minolta CR-200/300 spectrophotometer, with illuminant A and a 10° viewing angle and calibrated on a white tile. $L^*$, $a^*$ and $b^*$ values were recorded and chroma $[C^* = (a^{*2} + b^{*2})^{1/2}]$ and Hue $[h^\circ = \tan^{-1}(b^*/a^*)]$ were calculated. All data reported here were measured on the side with less ridges or irregularities.

### Visual quality

Overall visual quality was evaluated on a 9 to 1 scale, where 9 = excellent, no defects, 7 = good, minor defects, 5 = fair, moderate defects, 3 = poor, major defects and 1 = unusable. A score of 6 was considered the limit of salability. Dehydration and discolouration were evaluated on scales of 1 to 5, where 1 = none, 2 = slight, 3 = moderate (5–20% surface affected), 4 = moderately severe (20–50% surface affected) and 5 = severe (>50% surface affected).

### Respiration rates

Potato slices (150 g) were placed at 5 °C in polycarbonate containers connected to a flow of humidified air (c. 95% RH) controlled by capillaries to allow accumulation of $CO_2$ to 0.5%. The $CO_2$ concentrations were measured daily by taking 1 mL gas samples and injecting into an infrared analyser calibrated with a 0.5% $CO_2$ standard.

### PAL activity

Phenylalanine ammonia lyase activity was assayed as described by Ke & Saltveit (1986) with modification. Four grams of finely chopped tissue (without peel) was weighed into a plastic test tube containing 0.4 g soluble polyvinylpolypyrrolidone (PVPP), placed on ice and frozen at −80 °C. For analysis, 16 mL of 50 mM borate buffer (pH 8.5, containing 400 μLper 1000 mL 2-mercaptoethanol) was added, sample homogenised, filtered on ice, and centrifuged at 15 000 × ***g*** for 20 min. Two tubes of 5 mL supernatant were heated at 40 °C for 5 min, with aliquot of 0.55 mL of 100 mM L-phenylalanine added to one tube and mixed, and 0.55 mL water added to the other. Absorbance at 290 nm was measured, tubes incubated at 40 °C for 1 h, and $A_{290}$ remeasured. One unit PAL activity was defined as the formation of 1 μmol cinnamic acid in 1 h.

### PPO activity

Polyphenol oxidase analysis was based on the method described by Siriphanich & Kader (1985). Four grams of

finely chopped slices (without peel) was weighed into a plastic test tube containing 0.4 g insoluble PVPP, placed on ice and frozen at −80 °C. Sample was homogenised with 16 mL phosphate buffer (50 mM, pH 6.2), filtered, centrifuged at 12 000 × ***g*** for 20 min at 4 °C, and the supernatant used for analysis. To 933-μL enzyme extract was added 67 μL caffeic acid, and activity was determined by the increase in absorbance at 420 nm 3 min after adding the enzyme extract and with continuous shaking. 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.

### Phenolics

The determination of total soluble phenolics was based on a Folin–Ciocalteu method (Folin & Ciocalteu, 1927; Hyodo *et al*., 1978). Four grams of finely chopped tissue (without peel) was frozen at −80 °C. Before thawing, tissue was homogenised with 15 mL of 80% ethanol and filtered through Whatman no. 1 paper. For analysis, 0.25-mL extract was added to 2.5 mL Reagent C [1 part of Reagent A plus 98 parts of Reagent B, both freshly prepared; Reagent A is a 2.7% solution of sodium potassium tetrahydrated tartrate (w/v); Reagent B is 2.0% sodium carbonate (w/v) in 0.1 N sodium hydroxide] and let stand 10 min. Then 0.25 mL Reagent D (one part of commercial Folin–Ciocateu reagent and one part of water freshly prepared) was added, solution agitated vigorously and let stand 60 min. Absorbance at 760 nm was determined and calculations were based on a standard curve of 0–160 μg $mL^{-1}$ gallic acid.

Individual phenolics were determined by HPLC. One gram of freeze-dried potato (without peel) powder was homogenised 1 min in 20 mL methanol with naphthoic acid as internal standard. Extract was vacuum-filtered through Whatman no. 1 paper, rotary evaporated at 55 °C, re-dissolved in 5 mL MeOH, and membrane filtered before injection (10 μL). The HPLC system (Shimadzu Scientific Instruments Inc., Pleasanton, CA, USA) consisted of pumps (LC-10ADvp), autosampler (SIL 10-ADvp), column oven (CTO 10-Avp), diode-array detector (SPD-M10Avp) and controller (CBM-10AWvp), and was managed with EZ Start software (Shimadzu version 7.4 SP1 R2). Phenolic acid separation was done on a Luna C18(2) column (Phenomenex 100Å, 150 × 4.6 mm, 5 μm; Phenomenex, Torrance, CA, USA) and gradient elution, with a mobile phase (1 mL $min^{-1}$) consisting of 9:1 water:acetic acid (solution A) and methanol (solution B): isocratic elution 100% A/0% B, 0–15 min; linear gradient 90% A/10% B, 15–20 min; isocratic elution 80% A/20% B, 20–30 min; linear gradient from 50% A/50% B to 20% A/80% B, 30–35 min. Phenolics were identified at 280 nm and quantifications were based on peak area. Calibration curves of the standards were made by diluting stock solutions in methanol.

### Experimental design and statistical analysis

All experiments were conducted with three replicates per treatment (10 or 20 potato slices per replicate) in a completely randomised design. Data were analysed as averages ± standard deviation and/or by ANOVA with mean separation by LSD at $P < 0.05$ (SPSS SigmaStat 3.0; SPSS Inc., Chicago, IL, USA).

## Results and discussion

### Selection of potato cultivar

Potato cultivars can differ in browning potential. Fresh-cut slices of cv Pacific Russet were below the limit of salability by day 4, while slices from the California white skin potato were marketable after 12 days (Fig. 1). Russet potato cultivars were identified previously as having high browning potential compared with other cultivars (Sapers *et al*., 1989; Cabezas-Serrano *et al*., 2009). The cv Pacific Russet was used in all subsequent tests.

### Chemical treatments and respiration rates

A preliminary screening of chemical dips was conducted (data not shown). Chemicals that were effective to retard discolouration and are commonly used in fresh-cut processing included AA, CIA and sodium bisulphite (SB). AA (1.25%) or CIA (1.25%) dips used alone were ineffective, but their combination provided some benefit. SB dips (0.025–0.25%) were very effective to retard browning.

Respiration rates of slices treated with SB at 0.1% were similar to those of untreated slices, but 0.25% SB reduced respiration rates (Fig. 2), consistent with data of Petri *et al*. (2008). The AA + CIA treatment, however, resulted in respiration rates consistently higher than those of untreated slices (Fig. 2). This result agreed with previous reports (Limbo & Piergiovanni, 2007; Rocculi *et al*., 2007).

### Effect of controlled atmospheres on visual quality and colour

Two tests were conducted with a range of CA conditions to evaluate the control of browning on fresh-cut potato slices. Visual quality declined dramatically during the initial 4 days at 5 °C (Fig. 3). The $L^*$ and hue colour values decreased while $a^*$, $b^*$, and chroma values increased, all indicative of discolouration on the cut surfaces. Reducing $O_2$ from 21% to 3% or 0.3% had little effect on quality and colour. Potato slices in 12%

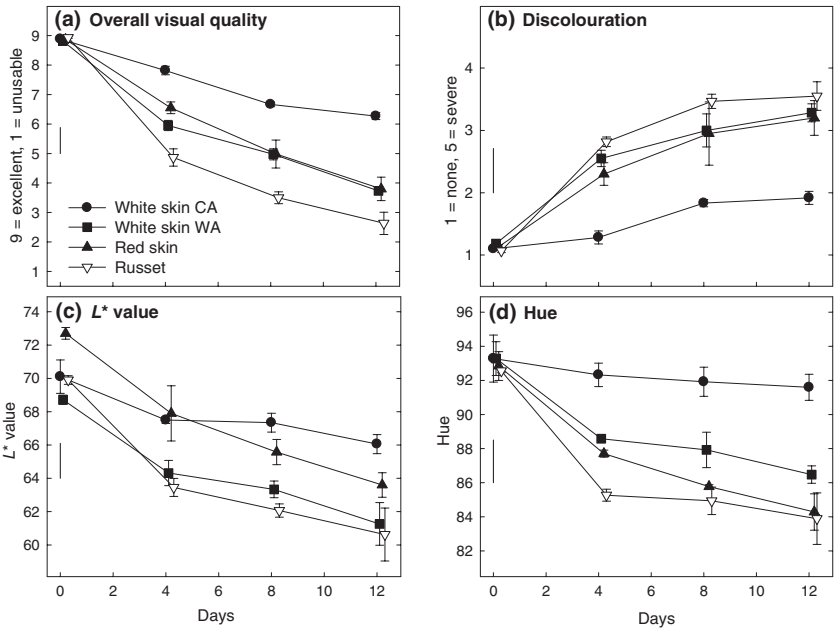


**Figure 1** Effect of potato source on the overall visual quality, discolouration and colour values of slices stored in air at 5 °C. A visual quality score of 6 is considered the limit for marketability. Potatoes cultivars were white flesh and white skin from California or Washington, red skin from Oregon or Russet potatoes from Washington. Data are averages of three replications ± standard deviation.

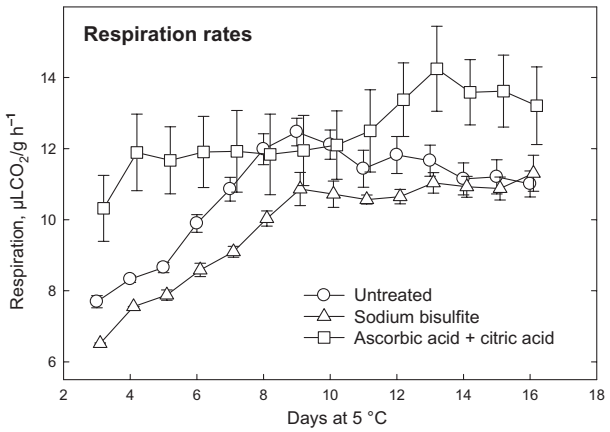


**Figure 2** Respiration rates of fresh-cut Russet potato slices at 5 °C. Slices were untreated or dipped in 0.25% sodium bisulfite or 1.25% ascorbic acid + 1.25% citric acid for 3 min. Data are averages of three replications ± standard deviation.

$CO_2$ with 0.3% or 3% $O_2$ had less browning and colour change and higher quality than those stored in 6% $CO_2$. An $O_2$ concentration as low as 0.3% would likely induce anaerobic metabolism (Mateos *et al.*, 1993; Gorny *et al.*, 2002; Angós *et al.*, 2007). Laurila *et al.* (1998b) found that modified atmospheres alone provided some benefit but did not completely maintain visual appearance of cut potato slices. The 3% $O_2$ + 12% $CO_2$ atmosphere was used in further testing because of the benefits noted and because it is an atmosphere that can be easily achieved with currently available plastic film packaging for fresh-cut products.

### Effect of controlled atmosphere and anti-browning chemicals on quality

When AA + CIA dip was combined with CA, browning and colour changes were retarded (Fig. 4a–c). Slices treated with SB (with or without CA) and AA + CIA (with CA) were still of marketable quality at day 8. This synergistic combination of AA + CIA with CA has been reported for various fresh-cut products (Laurila *et al.*, 1998a; Gorny *et al.*, 2002; He & Luo, 2007), but Jiang *et al.* (2004) found that CIA alone retarded discolouration of cut water chestnut. SB was much better than AA + CIA as an anti-browning agent, a result consistent with many other reports (Gunes & Lee, 1997; Laurila *et al.*, 1998b; Beltrán *et al.*, 2005). AA or/and CIA have been found effective to inhibit browning on cut potato in other studies (Ahvenainen *et al.*, 1998; Rocha *et al.*, 1998; Jiang *et al.*, 2004; Limbo & Piergiovanni, 2006, 2007), but were ineffective in the present study on high browning potatoes.

Low concentrations of SB were effective to retard surface discolouration of fresh-cut potatoes (Fig. 5). The 0.25% SB treatment maintained initial $L^*$ and hue colour values, whereas the 0.025% treatment was not as effective. In the two experiments, a 0.025% dip was less effective than dips in 0.05%, 0.075%, 0.1% or higher concentrations. Visual quality, discolouration, and colour values were similar among SB treatments of 0.05%, 0.075%, 0.1% or higher (data not shown). An atmo-

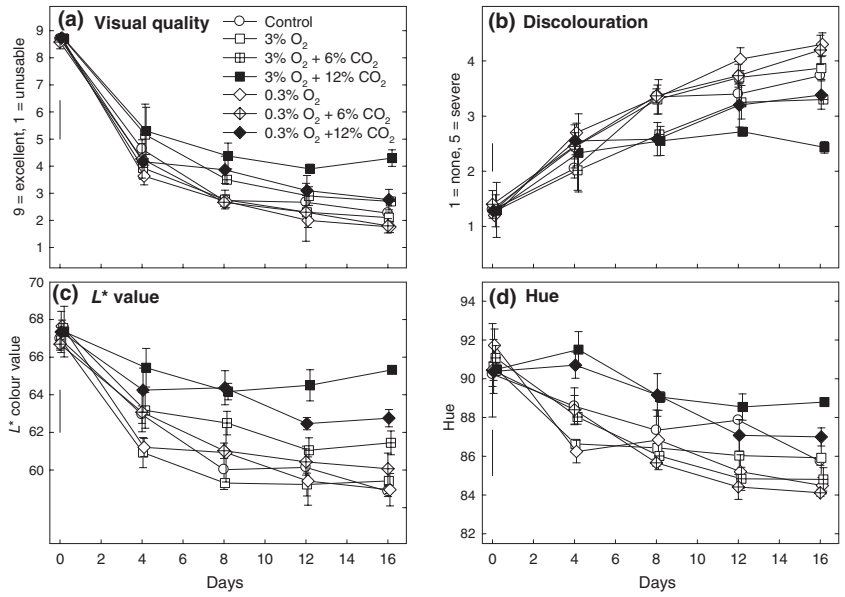


**Figure 3** Effect of controlled atmospheres on overall quality, discolouration, and colour values of fresh-cut Russet potato slices stored in air or controlled atmospheres at 5 °C. Data are averages of three replications ± standard deviation.

sphere of 3% $O_2$ + 12% $CO_2$ provided additional benefit to quality of the slices treated with 0.025% SB but little additional benefit to slices treated with 0.25% SB (Fig. 5). The effective SB concentrations were generally lower than those reported effective by other authors or those used in the fresh-cut potato industry (Gunes & Lee, 1997; Laurila *et al*., 1998b; Beltrán *et al*., 2005; Food Marketing Institute (FMI), 2010).

Potato slices treated with SB had little browning, regardless of the storage atmosphere. However treatment with SB (0.25%) resulted in a noticeable yellowish appearance of the cut slices at the end of storage. These slices had the greatest increases in $b^*$ and chroma colour values (Fig. 5d), and this may be due to the formation of intermediate chlorogenic quinones which can form yellow solutions (Murata *et al*., 2002). In another test, SB concentrations of 0.1%, 0.25% and 0.5% all inhibited potato discolouration and changes in $L^*$ and $a^*$ colour values, but again there were increases in $b^*$ and chroma values (data not shown). Use of sulphites can lead to sensory problems (Beltrán *et al*., 2005), but very low SB concentrations (0.025–0.05%) should have no impact on sensory attributes of potatoes while providing excellent control of browning (Laurila *et al*., 1998b).

Dehydration on air-stored slices was slightly higher at the end of storage (score of 3) than on CA-stored slices (score of 2). However, the effects of dehydration were minor compared with the impact of discolouration on visual quality. In all experiments visual quality scores were highly correlated with discolouration but not dehydration scores (data not shown). Chemical treatments did not result in differences in dehydration scores compared with scores for control slices.

### Effect of controlled atmosphere and anti-browning chemicals on PAL and PPO activities and total phenolics

Tissue wounding leads to induction of synthesis and activity of PAL, the first committed enzyme of phenylpropanoid metabolism, and its activity is often correlated with accumulation of phenolic compounds and tissue browning (Saltveit, 1997; Peiser *et al*., 1998; Aquino-Bolaños *et al*., 2000). PAL activity was higher in slices AA + CIA-treated slices than in slices from other treatments (Fig. 4d). PAL activity of untreated and SB-treated slices in air were similar over 16 days, while PAL activities were suppressed in slices from the corresponding treatments stored in CA. These results are consistent with other reports of PAL inhibition by modified atmospheres (Salman *et al*., 2008; Chen *et al*., 2009).

The activity of PPO was similar in slices from all treatments, with slightly higher activity in the air-stored slices than CA-stored slices at day 12 and 16 (Fig. 4e). Sapers *et al*. (1989) found that PPO activity was correlated with browning in potato cultivars, while Cantos *et al*. (2002) found no relationship between PPO activity and browning. PPO activity was not much affected by the chemical treatments in this study, but others have reported decreases or increases in PPO activity depending on the concentrations of ascorbate and citrate (Pizzocaro *et al*., 1993; Jiang *et al*., 2004; Zhu *et al*., 2009).

Total phenolics increased in air-stored potato slices, regardless of chemical treatment, throughout the 16-day period (Fig. 4f). Increases in phenolics were retarded by CA, regardless of chemical treatment and

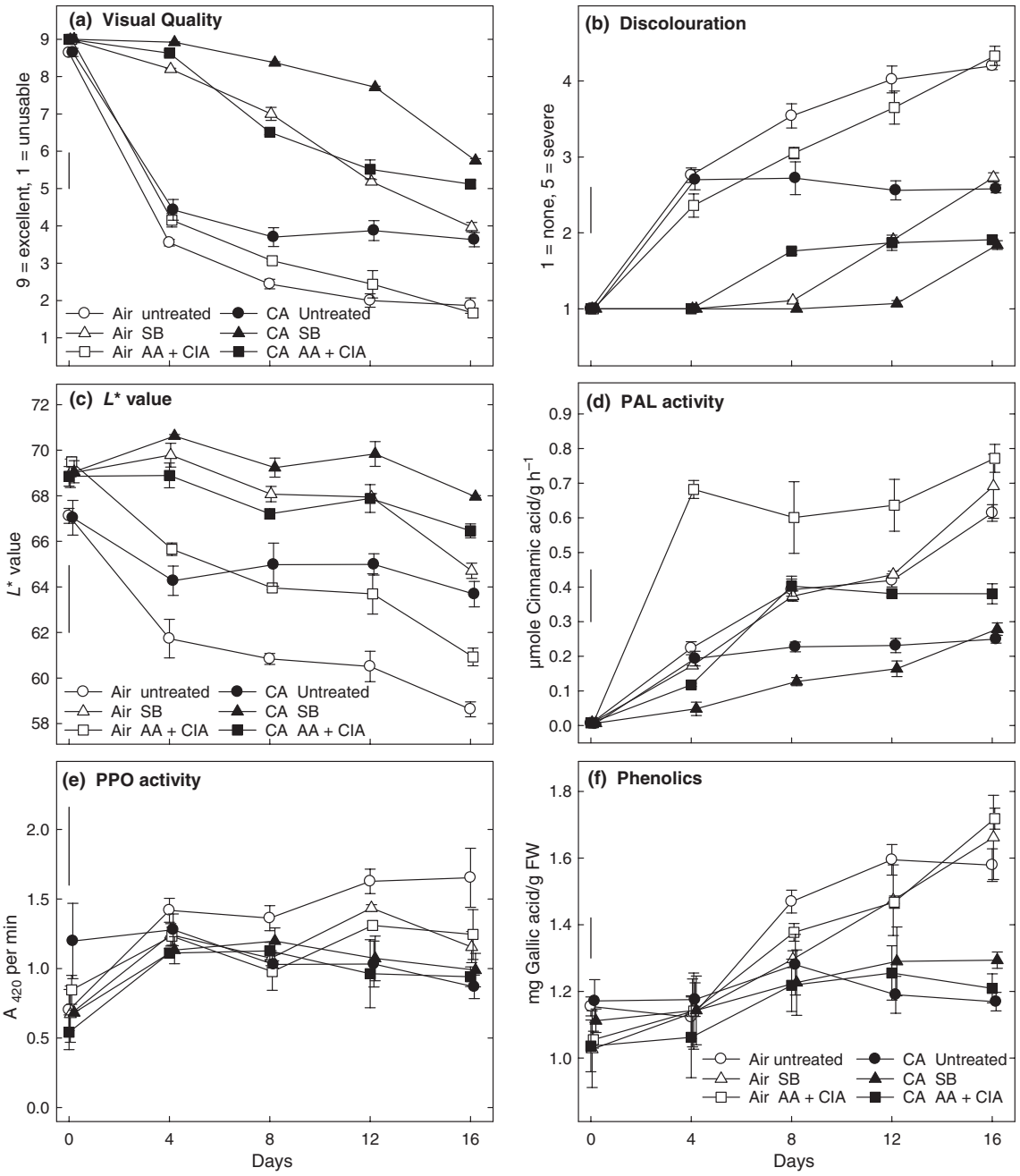


**Figure 4** Visual quality, discolouration, $L^*$ colour values, phenolic concentrations, phenylalanine ammonia lyase activity and polyphenol oxidase activity of fresh-cut Russet potato slices stored in air or controlled atmosphere (3% $O_2$ + 12% $CO_2$) at 5 °C. Slices were untreated or dipped for 3 min in 0.25% sodium bisulfite (SB) or 1.25% ascorbic acid and 1.25% citric acid (AA + CIA). Data are averages of three replications ± standard deviation. Vertical bars indicate LSD 0.05.

particularly from day 8 onwards (Fig. 4d). In cut peaches, Zhu *et al.* (2009) reported that a 0.2% AA treatment resulted in increases in total phenolics, although PPO and peroxidase activities were inhibited.

## Concentrations of individual phenolic acids in fresh-cut potato

In unwounded potato tissue, caffeic, gallic, chlorogenic and protocatechuic acids occurred in low concentrations

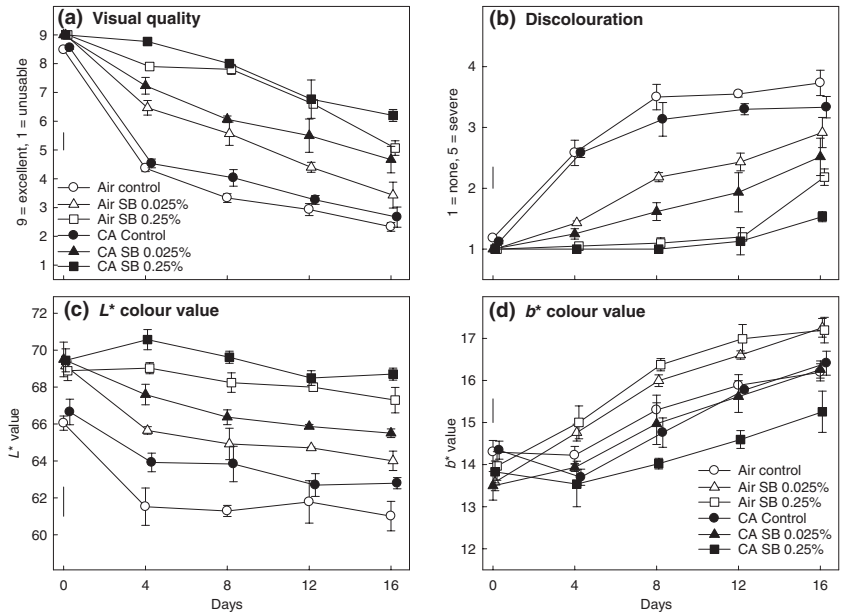


**Figure 5** Visual quality, discolouration, $L^*$ and $b^*$ colour values of fresh-cut Russet potato slices stored in air or controlled atmosphere (3% $O_2$ + 12% $CO_2$) at 5 °C. Slices were untreated or dipped for 3 min in 0.025% or 0.25% sodium bisulfite (SB). Data are averages of three replications ± standard deviation. Vertical bars indicate LSD 0.05.

(Fig. 6). By 4 days after cutting there was an increase in the concentrations of all four phenolic acids. Chlorogenic acid (3-*o*-caffeoylquinic acid) became the predominant phenolic acid after 8 and 12 days. In another test, chlorogenic acid concentrations were similar at 8 and 12 days (data not shown). In both cases, however, chlorogenic acid concentrations had decreased by day 16. Chlorogenic acid concentrations were lower in CA-stored than air-stored slices regardless of chemical treatments.

Chlorogenic acid may comprise up to 90% of the phenolic acids in cut potato at concentrations similar to those reported here (Friedman, 1997). Chlorogenic acid is a very good substrate for PPO, followed in activity by catechol, catechin, caffeic acid and gallic acid, respectively. Chlorogenic acid is oxidised by PPO to a highly reactive *o*-quinone intermediate which can then interact with $NH_2$ groups of lysine, SH groups of cysteine, $SCH_3$ groups of methionine and other reactive groups in the browning reactions in potato (Friedman, 1997).

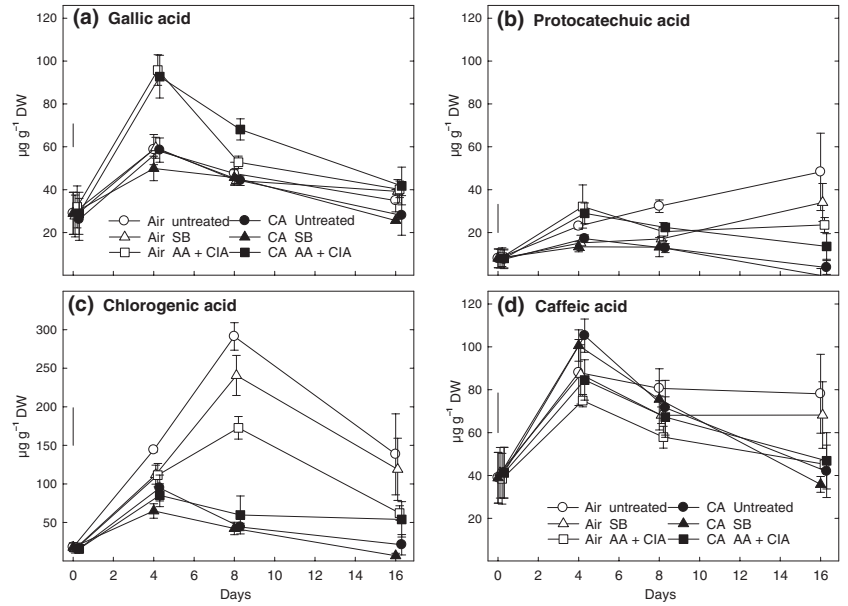


**Figure 6** Individual phenolic concentrations of fresh-cut Russet potato slices stored in air or controlled atmosphere (3% $O_2$ + 12% $CO_2$) at 5 °C. Slices were untreated or dipped for 3 min in 0.25% sodium bisulfite (SB) or 1.25% ascorbic acid and 1.25% citric acid (AA + CIA). Data are averages of three replications ± standard deviation. Analyses were from the same experiment described in Fig. 4.

Caffeic and gallic acids were induced by wounding with maximum threefold increases by day 4 (Fig. 6). The highest concentrations were found in the control and SB treatments stored in CA, while the lowest concentrations were in AA + CIA-treated slices in air or CA. Protocatechuic acid had the lowest concentration among the four phenolic acids, and after an initial increase, concentrations were suppressed by CA.

### Time course of browning and phenolic metabolism

Phenolic metabolism and polymerisation is required for browning of fresh-cut potato (Friedman, 1997). Despite differences in brown discolouration of cut slices among four cultivars (Fig. 1), changes in phenolic concentrations were similar (data not shown). Nor were temporal or concentration changes in phenolic acids related to browning of fresh-cut potato in tests with controlled atmospheres and browning inhibitors (Figs 4 and 6). The lack of direct relationship between changes in phenolics and enzymatic browning of cut potatoes has been previously reported (Cantos *et al*., 2002), while others have reported a closer temporal correlation in potato (Sapers *et al*., 1989; Thybo *et al*., 2006) and other root (Aquino-Bolaños *et al*., 2000) or fruit crops (Chen *et al*., 2009). This apparent discrepancy may reside in the fact that soluble browning pigments were analysed in tissue samples while insoluble pigments causing discolouration were measured on the cut surfaces. More likely, the physical damage caused by cutting allows mixing of phenolic substrates with enzymes and that alone is sufficient to cause rapid browning; changes in concentrations or activities may not be needed (Cantos *et al*., 2002; Toivonen & Brummell, 2008).

## Conclusions

The browning susceptibility of fresh-cut slices differed among potato varieties. Controlled atmospheres only slightly retarded quality loss of fresh-cut potato, but were effective in suppressing wound-induced PAL activity and increases in phenolic compounds. AA + -CIA (1.25%), although not effective alone, provided good browning control when combined with CA. SB dips as low as 0.025% retarded discolouration. Although this concentration was not as effective as 0.05% or higher, it was very effective when combined with moderate CA (3% $O_2$+ 10%$CO_2$). Chlorogenic acid was the major phenolic acid and concentrations increased markedly after cutting. CA inhibited accumulation of phenolics acids but chemical treatments did not. There was no temporal relationship between the onset of browning of cut potato slices and changes in phenolic enzymes or phenolic acid concentrations.

## Disclosure

No competing financial interests exist.

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