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Effects of postharvest curing treatment on flesh colour and phenolic metabolism in fresh-cut potato products

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ABSTRACT

The flesh colour and phenolic metabolism in potato tuber during curing and after cut were investigated. Result indicated that postharvest curing not only changed phenolic metabolism during curing, but also improved fresh-cut colour for 12 days after fresh cut. Significantly lower PAL and higher phenolic content and PPO activities during curing treatment and fresh-cut potatoes were detected compared to the control, which lead to the lower browning in the slices from curing treated potatoes. HPLC analysis revealed that amounts of total phenolics, chlorogenic acid, gallic acid and protocatechuic acid were induced by curing and highly accumulated in the curing treated potatoes. Our results demonstrated that phenolic metabolism played an important role in the control of browning of fresh cut potato after curing.

1. Introduction

Potato (*Solanum tuberosum*) is the third largest food crop in world. In 2010, the worldwide production of potatoes reached 324 million tones. As global living standards increase, so too is the demand for fresh-cut potatoes. However, just like many fruits and vegetables, fresh-cut potatoes are prone to browning after cut. This turns out to be the major limitation for their shelf life (Cantos, Tudela, Gil, & Espín, 2002; Ma, Wang, Hong, & Cantwell, 2010; You et al., 2012) and prevents fresh-cut potatoes from being a more popular consumer choice. Browning may be the symptom of an ongoing degenerative process such as the damaging of cell compartmentalization (Marangoni, Palma, & Stanley, 1996), as well as the interaction of phenolic compounds (substrates) and polyphenol oxidases (PPO) that are activated after the cut surface contacted with oxygen (Degl'Innocenti, Pardossi, Tognoni, & Guidi, 2007). Browning may also be the result of an active inductive process, requiring de novo synthesis of PAL and the consequent accumulation of phenolic compounds (Saltveit, 2000). Although high phenolic compound activities are associated with high potential of browning (Thybo, Christiansen, Kaack, & Petersen, 2006), they also have positive benefits such as enhancing the antioxidant capacity of plant tissue, mainly related to its role of eliminating reactive oxygen species (ROS) and free radicals (Rechner, Pannala, & Rice-Evans, 2001). Many polyphenols, especially phenolic acids, are directly involved in the response of plants to different types of stress. These chemicals contribute to healing by lignifications of damaged areas, and possess antimicrobial properties by increasing concentrations after pathogen infection. Chlorogenic acid and caffeic acid are highly accumulated in potato peel, rather than in the flesh inside of the potatoes. Phenolic oxidation reactions alter the quantity of polymerized substances, which in turn directly impacts the quality of foods, particularly in colour and organoleptic characteristics. Such changes may be beneficial (as is the case with black tea) or undesirable (browning of fruit) to consumer acceptability. For fresh-cut potatoes, a correlation between browning and PAL activity is found only during the first 4 days after wounding (Cantos et al., 2002). No significant correlation was found between either rate or degree of browning and PPO, POD and total or individual phenolics. The mechanism of browning in potato tuber fresh-cut is still very obscure.

Various approaches have been applied to extend the shelf life of fresh-cut potatoes. These methods include use of chemical compounds and plant extracts (Oms-Oliu et al., 2010), as well as modified atmosphere packaging that exclude oxygen from the environment (Kang & Saltveit, 2003; Ma et al., 2010). However, these anti-browning means are generally constrained due to their high cost, low efficiency or potential health hazards. Therefore, it is

Abbreviations: PAL, phenylalanine ammonia lyase; PPO, polyphenol oxidase; 4Cl, 4-coumarate coenzyme A ligase; C4H, cinnamate 4-hydroxylase; CK, control; EL, electrolyte leakage; PVPP, polyvinyl polypyrrolidone; NaOCl, sodium hypochlorite.

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desirable to develop a simple, safe and cost-effective method to extend the shelf life of fresh-cut potatoes for commercial use.

Curing is a normal practice after potato harvested to promote dormancy and extend postharvest storage life, by preventing decay caused by microorganism during storage (Hide & Cayley, 1983, 1987). Curing at 15 °C for 14 days in dry conditions reduced the incidence of skin spot from 70% prick wounds infected down to 4%. In damp conditions, however, curing only reduced the problem down to 53% (Hide & Cayley, 1987). Kim and Lee (1992) reported that reconditioning of potato improved chip colour by reduce no enzymatic browning during high temperature frying. However, the biochemical changes related to phenolic metabolism and enzymatic browning in cured potato tuber flesh and after cutting remains unclear.

To date, no information is available on the effect of postharvest curing treatment on fresh-cut potatoes in terms of colour, detailed physiological and biochemical changes. Accordingly, the objective of the present study is to investigate the effect of postharvest curing on the colour of fresh-cut potatoes, and to understand possible mechanism by measuring respiration, membrane leakage, PAL, PPO activities and phenolic content during curing and the subsequent fresh-cut shelf life period.

2. Materials and methods

2.1. Plant material and curing treatment

Potatoes (*S. tuberosum, cv* Netherlands #7) were purchased from freshly harvested local wholesale market, Tai'an, Shandong Province, China. After transported to the laboratory, non-damage and non-defective tubers were selected for 10-days curing treatment and then used for fresh-cut experiment. The potato fresh cut experiments using curing treated potato tubers were repeated for four years from 2010 to 2013. The flesh colour and phenolic metabolism changes related to browning were investigated with freshly harvested potatoes both during curing of intact potatoes and after curing period for fresh-cut potatoes in the year of 2013, in which the individual phenolic compounds were analysed by High Performance Liquid Chromatography (HPLC). The results of different experiments in different years were very similar in colour value, overall visual quality, and PPO, PAL activity of fresh-cut potatoes. The data presented here were from the results of 2013 to make sure all information comes from same plant materials.

Curing treatment: Potatoes were packed in Polyethylene (PE) plastic bags and put in a thermostat-controlled cabinet for 10 days. One group was stored at 16 ± 1 °C for curing, while the other group was stored at 2–3 °C (commercial storage temperature in China) as control (CK). Three samples were taken at 0, 5, 10 days, during curing treatment period.

2.2. Fresh cut experiment

After 10 days curing treatment, potato tubers were removed from both temperature storage (curing and control) and used for fresh-cut experiment. The tubers were hand-peeled and cut into 5 mm thick slices, which were immediately rinsed in 50 ppm NaOCl (pH 7.0) for 5 min. The excess water was removed by draining and blotting with cheesecloth, and the slices were packed into PE bags, stored at 2–3 °C. The slices were removed from cold storage after 0, 3, 6, 9 and 12 days. Randomly selected individual slices (8 pieces) per replicate and 3 replicates per sample were collected and analyzed. The analysis included determination of colour change, respiration, and conductivity as well as total and individual phenolic compounds, PPO, PAL enzyme activities.

2.3. Visual quality assessment

Visual quality was examined in accordance with the sensory evaluation standards (Ma et al., 2010). The overall visual quality was evaluated on a 9–1 scale, with 9 as freshly cut, equaled to excellent, with no defects; 7 as very good, with minor defects; 5 as fair, with moderate defects; 3 equaled poor, with major defects; and 1 indicated inedible. A score of 5 was considered the limit of salability and shelf-life was defined at the days required to reach a score of 5.

2.4. colour measurement

The surface colour of slices was determined with a Minolta CR-400 colourimeter. The L^*(lightness), a^* (reddish–greenish) and b^* (yellowish–bluish) indexes of the CIELAB colourimetric system were used to evaluate the colour change of the potato samples. Each slice was measured twice (each side), 8 individual slices from each replicate were measured. (16 measurements were carried out for each replication and three replications for each treatment at each time point.)

2.5. Enzyme assays

Randomly selected potato slices were collected at 0, 3, 6, 9, 12 days after fresh cut and frozen immediately by liquid nitrogen. The samples were then grinded with liquid nitrogen into frozen powder by an analytic mill (IKA A11 basic; IKA Werke GmbH & Co. KG, Staufen, Germany), and stored at −80 °C until used. PPO activity was analysed based on the method described by Galeazzi, Sgarbieri, and Constantinides (1981). One g of frozen powder was homogenized with 4 mL of phosphate buffer (pH 7.0) and 0.1 g insoluble polyvinylpolypyrrolidone (PVPP), centrifuged at 10,000×*g* for 15 min at 4 °C, and the supernatant was used for analysis. The reactive mixture consisted of 0.1 ml of enzyme extracts, 2 ml of 200 mM phosphate buffer (pH 6.8) and 0.5 mL of 20 mM catechol solution. Enzyme activity was measured by the increase in absorbance at 410 nm. One unit of enzyme activity was defined as the increase in absorbance of 0.01 per min under assay conditions. PPO activity was expressed as unit of activity per mg protein per h. Protein content was measure as described by (Bradford, 1976) from the same extraction buffer at pH 7.0.

PAL activity was measured as previously described by Martinez-Tellez and Lafuente (1997), with slight modifications. 1 g frozen powder described as above PPO assay was homogenized with 4 mL of 50 mM borate buffer (pH 8.5), centrifuged at 10,000×*g* for 15 min at 4 °C. 2 ml buffer (pH 8.5) and 1 ml of 20 mM L-phenylalanine was added to two individual tubes, 0.3 ml of enzyme solution (from supernatant) was added to one of the tubes and 0.3 ml water was added to the other tube. Absorbance at 290 nm was measured twice (before and after reaction tubes were incubated at 40 °C for 1 h). One unit of PAL activity was defined as the amount of enzyme produced as an increase of 0.01 absorbance units in 1 h. PAL activity was expressed as unit of activity per mg protein per h.

2.6. Determination of phenolic

HPLC method was used for individual phenolic compounds analysis. The phenolic compounds were extracted according to the method (Zhou, Zeng, Shi, & Xie, 2008) with minor modifications. 0.2 g frozen flesh powder obtained in same method as enzyme assay were vortexed with 0.8 ml methanol (MeOH, 80%, formic acid 1%) then extracted over night at 4 °C in refrigerator. The extraction mixture was sonicated at 30 °C for 30 min, and centrifuged at 10,000×*g* for 30 min. The residue was re-extracted with

0.5 ml 80% methanol and centrifuged again. Two extraction solutions were combined and filtered through a 0.45 μm syringe filter prior to HPLC analysis. Stock solutions of phenolic standards were prepared in 80% methanol with 1% acetic acid (v/v). All solvents were filtered through 0.45 μm membranes and degassed by vacuum filtration before being used.

Separation of phenolics was performed using a reversed phase column (inertsil ODS-3, 5 μm, 4.6 × 150 mm, C/N 5020-01731, GL Science Inc. Japan). The mobile phase was A: 1% acetic acid in filtered distilled water; B: 100% HPLC grade methanol. The gradient program used was as follows: 0–5 min: 10% B, 5–15 min: linear gradient 10–20% B, 15–30 min: linear gradient 20–40% B, 30–35 min: 40–50%, 35–45 min: 50–80%, 45–55 min: 80–10%, 60 min: stop. The flow rate was 0.8 ml/min and injection volume was 10 μl for the standard, 60 μl for sample. The system operated at a temperature of 40 °C. Peaks were identified by comparison of retention time and UV spectra with authentic standards. The concentration of individual phenolic compounds was determined based on peak area and calibration curves derived from corresponding authentic compounds. The total phenolic content was determined as the summary of 6 peak areas from 280 nm at retention time (RT) 3.4, 5.4 and 11 min and 326 nm at RT 12, 22, and 34 min, respectively, converted to phenolic content (μg/g FW).

2.7. Determination of respiration rates

The potato slices (200 g) were placed in plastic containers and sealed for 12 h at 3 °C storage temperature. The CO_2 concentration was measured using CO_2 instrument (PBI-940437B, PBI Dansensor, Denmark). The respiration rate was calculated as described previously by Castelló, Fito, and Chiralt (2006).

2.8. Experimental design and statistical analysis

All experiments were conducted with three replicates per treatment in a completely randomized design. Data were analysed by ANOVA with mean separation and LSD at $P < 0.05$. All data represented in the figures were the means of three replicates ± standard deviation.

3. Results and discussion

3.1. Effects of curing treatment on flesh colour of intact potato, slices colour and quality of fresh cut potato

Curing is a practice in potato and food industry to cure the potato skin periderm after harvest and prevent decay loss during storage. Here, the effects of postharvest curing treatment on fresh-cut potatoes were investigated. The flesh colour of intact potato and juice colour were significantly affected by curing treatment compared to the control stored at conventional storage temperature (Figs. 1A,C and 3B). Among three colour indexes (colour L, a, b), potato flesh colour L^* and a^* values were significantly changed by the curing treatment. Higher "*L*" (Fig. 1A) and lower "*a*" value (Fig. 1C) and relative stable "*b*" colour (Fig. 1E) in the 10 days-treated potato tuber flesh indicated that curing temperature increased brightness of flesh colourcolour significantly.

During 12 d shelf life after fresh cut, the colour of potato slices from curing treated potatoes were consistently much better than those from control (Figs. 1B,D, and 3A). Significantly lower levels of browning of potato slices and better quality in overall visual quality from the curing-treated potatoes than control slices ($P < 0.05$) was observed (Fig. 2). Visual quality declined gradually after fresh cut to 12 d at 3 °C. This is correlated well with the decrease of "*L*" and the increase of "*a*" value in both curing and control. Higher "*L*" and lower "*a*" value were found in freshly cut slices from the cured potato throughout the 12 d shelf life period (Fig. 1B,D). Better "*b*" value in the slices from curing potato than those in the control potato also indicates the less browning occurred (Fig. 1F). The data above indicated that the curing treatment was effective in the preventing surface browning and maintaining visual quality of fresh-cut potatoes.

3.2. Total phenolic content and PAL PPO activities during curing and post cut storage time

To understand the biochemical bases for the curing effects, total phenolic content and PAL, PPO activities were measured. During curing, the difference in total phenolic content between curing treatment and control (Fig. 4A) were similar to the colour "*L*" data (Fig. 1A). However, PAL activities decreased from the day 0 to the day 10 in both curing and control samples. PPO activity was declined rapidly from the day 0 to the day 5 in control samples. No significant PPO activity changes were detected from the day 0 to the day 10 curing samples. Relatively lower PAL activity (Fig. 4C) and higher PPO activity (Fig. 4E) were detected in the samples from 5 to 10 days after curing compared to the control. Interestingly, higher PAL and the decreased PPO activity were associated with the decreased total phenolic content in the control potato tissue, while relative lower PAL activity and higher PPO activity were associated with increased total phenolic content in the curing potato. The results imply that the higher total phenolic content in the curing tissue is unlikely resulted from the more synthesis of new phenolic compounds through PAL pathway *in vivo*.

The slices from curing treatment had higher phenolic content except on day 9 compared to the control (Fig. 4B). However, a slow increase of total phenolic content was observed in control from the day 0 to the day 6, a rapid increase on the day 9 and drop on the day 12. PAL activity increased from day 0 to day 12 in slices from both curing and control potatoes. The rate of increase was similar from day 3 to day 12 (Fig. 4D). Similar to the precutting during the curing treatment period, PAL activity was suppressed in the fresh-cut slices from the curing treatments from day 3 to day 12 compared with control. The maximum difference is about 30% higher in the slices from control than those in the curing treated samples on the day 12 after cut. The trend of PPO activity, on other hand, was highly correlated with phenolic content (Fig. 4B,F). Almost a doubled PPO activity was found on the day 9 to the day 12 in the slices from curing treated potatoes than those from the control. These biochemical differences between curing and control lead to a significant difference of browning score. It was reported that tissue wounds leads to induction of synthesis and activity of PAL, which is often correlated with accumulation of phenolic compounds and tissue browning (Aquino-bolanos, Cantwell, Peiser, & Mercado-silva, 2000).

3.3. Individual phenolic compounds profiles during curing and fresh cut shelf life period

Authentic compounds gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid and other chemical standards were separated in same HPLC program as external standard. Peaks were identified by comparison of retention time (RT) and UV spectra with authentic standards (Supplemental Fig. 3). The concentration of individual phenolic compounds was determined based on peak area and calibration curves derived from corresponding authentic compounds. The six major phenolic compounds were detected in 80% methanol (plus 1% acetic acid) extracts. Gallic acid, protocatechuic acid and chlorogenic acid were identified throughout the experiment period. Our data demonstrated that the curing not only significantly changed potato flesh colour (Fig. 1A & C

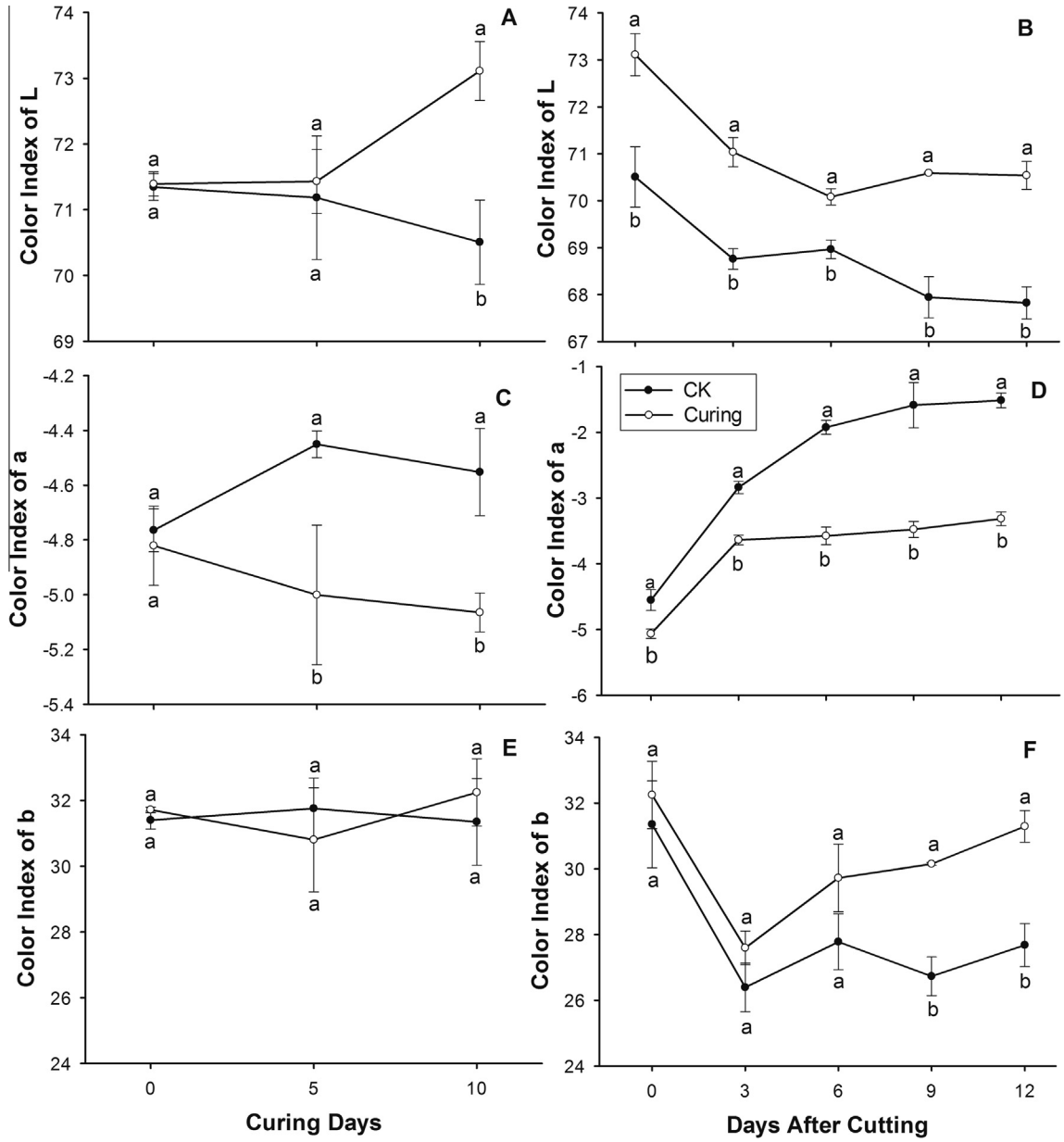

Fig. 1. (A–F) Effects of postharvest curing treatment on potato flesh colour (L^*, a^*, b^* colour) during the curing and after cutting. (A) Colour value "L" during curing; (B) colour value "L" after cutting; (C) colour value "a" during curing; (D) colour value "a" after cutting; (E) colour value "b" during curing; (F) colour value "b" after cutting, respectively. Data are the averages of three replications ± standard deviation. Each replicate has 6 potatoes. Test of the significance of difference are shown, "a" vs "a" meaning there is no significant difference ($P > 0.05$); "a" vs "b" meaning there is significant difference ($P \leqslant 0.05$).

and phenolic metabolism before cut, but also dramatically affected phenolic metabolism of fresh cut slices during 12 d storage time at 3 °C (Fig. 5A–F). No caffeic acid or little coffee acid was detected in the slices of potato variety Netherlands 7 either before or after wounding comparing with standard HPLC absorbent pattern and RT (Supplemental Figs. 1 and 3). Protocatechuic acid was the predominated phenolic compound in unwounded tissue (during the curing) and decreased during the curing period (Fig. 5A). The rapid decrease of protocatechuic acid in wounded tissue was observed 3 d after cut, and then increased on the day 9 and day 12 of the control samples; in the curing treated samples, however, it declined continually from the day 0 to day 9 (Fig. 5B). Chlorogenic acid was the second highest phenolic compound in unwounded tissue (Fig. 5C & supplemental Figs. 1, 2A). In the curing treated potato fresh-cut slices, amounts of chlorogenic acid dramatically increased from day 0 to day 12, almost doubled on the day 12 compared to the day 9 samples (Fig. 5D & Supplemental Fig. 1). In slices from control, however it slowly increased from the day 0 to day 6, peaked on day 9, and dropped until the day 12 (Fig. 5D & Supplemental Fig. 1), account to the largest phenolic compound on the day 9 in the fresh cut of control potato. Gallic acids were induced by wounding with maximum 6 fold increases by the day 6 for control and the day 9 for the curing treated slices. The patterns of changes were different in the slices from control or curing (Fig. 5F & Supplemental Fig. 1). An unidentified phenolic compounds at 326 nm (RT 12 min, data not shown) increased from the day 0 to the day 9 in both curing and control samples, rapid declined in the control slices but no changes on the day 12 in the curing treatment. The trends of chlorogenic acid, gallic acid and the unknown phenolic compound (RT 12 min) matched well with the trend of total phenolic content. The reduced large amount of these compounds on the day 12 control slices may be resulted from polymerization of phenolic compound at RT 35 min (Supplemental Fig. 1 Peak 4). However, on Russet potatoes, no temporal or

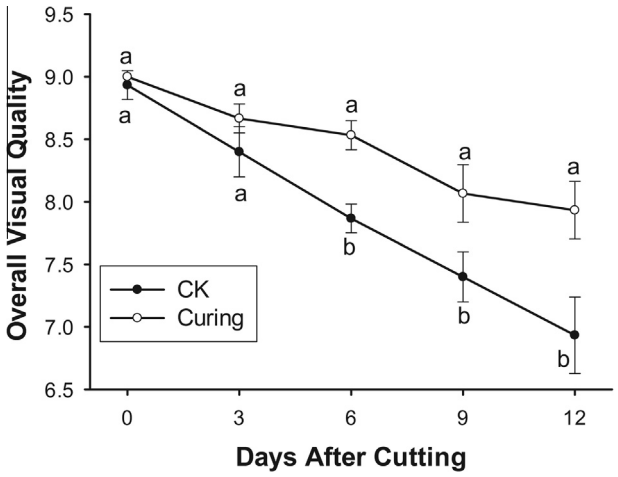

Fig. 2. The overall visual quality of fresh cut potato at 0, 3, 6, 9, 12 d after cut. Curing: potatoes were stored at the curing temperature for 10 d, CK: control from normal storage temperature at 3 °C for 10 d, and then used for cutting. Data are the averages of three replications ± standard deviation. Each replicate contains 8 pieces of slices. Test of the significance of difference is shown in the figure, "*a*" vs "*a*" meaning there is no significant difference ($P > 0.05$); "*a*" vs "*b*" meaning there is significant difference ($P \leqslant 0.05$).

concentration changes in phenolic acids related to browning of fresh cut potato in tests with CA and browning inhibitors were reported (Ma et al., 2010). The diversity of polyphenols makes their contents in food hard to estimate, including structural diversity, lack of standardized analytical methods and variation of content. The extraction and HPLC method used here not only gives a better estimation of total phenolic content, but also may provide the detail of flavonoids metabolic profile during the browning processing by a small extraction volume and allow a fast detection.

3.4. The relationship between phenolic content, PPO and PAL activity and association with tissue browning

Polyphenolic compounds are very important constituents because of their antioxidant activity in chelating redox-active metal ions, inactivating lipid free radical chains and preventing hydroperoxide conversion into reactive oxyradicals (Oliveira et al., 2009). An important function of phenolic acids is their action in plant defense mechanisms. Stress conditions such as excessive UV light, wounding or pathogen infection induces the biosynthesis of phenolic compounds. The phenylpropanoid pathway for the conversion of amino acid L-phenylalanine to trans-cinnamic acid by the PAL and the subsequent reactions producing new compounds such as chlorogenic acid, protocatechuic acid, caffeic acid and dicaffeoyltartaric acid are well known (Tomás-Barberán, Gil, Castaner, Artés, & Saltveit, 1997a; Tomás-Barberán, Loaiza-Velarde, Bonfanti, & Saltveit, 1997b). In our study, the increased phenolic contents in wound tissue are associated with stimulated PAL activity from day 3 to day 12 after cut. The rapid increase of phenolic content from day 3 to day 9 in control should have benefited from consist higher PAL activity than in curing samples at the same period. The decreased gallic acid, chlorogenic acid and other phenolic on day 12 could be the result of higher browning and polymerization in control slices. The PPO activity curves, however, are matched well with total phenolic curves and individual phenolic profiles in the same tissue. An increase of the storage time and PPO activity has been reported in 'Jonagored' apple slices (Rocha & Morais, 2002). This could be due to the cutting-induced increase in synthesis or activity of PPO (Kang & Saltveit, 2003).

A closer temporal correlation of phenolic concentration and browning in potato (Sapers, Garzarella, & Pilizota, 1990; Thybo et al., 2006) and jicama (Aquino-bolanos et al., 2000) or fruit crops (Chung & Moon, 2009) has been reported. No temporal or concentration changes in phenolic acids were found related to browning of fresh-cut potato in tests of browning inhibitors (Ma et al., 2010). Differences in brown discolouration of cut slices among different cultivars are not correlated with changes in phenolic concentrations. Yang, Zhou, Wu, and Cheng (2010) reported that sodium nitroprusside treatment inhibited PAL activities of peeled bamboo shoots, thus delayed external browning during storage. Saltveit (2000) reported that heat shock of iceberg lettuce leaves prevents an increase in PAL activity and browning. Rolle and Chism (1987) suggested that the level of PPO activity may be considered as an index for predicting susceptibility to browning. Antisense and sense mRNA of PPO affected tissue browning in potato tissue (Coetzer, Corsini, Love, Pavek, & Tumer, 2001; Steffens & Zabeau, 1994). Luna et al. (2012) found that low PPO activity was

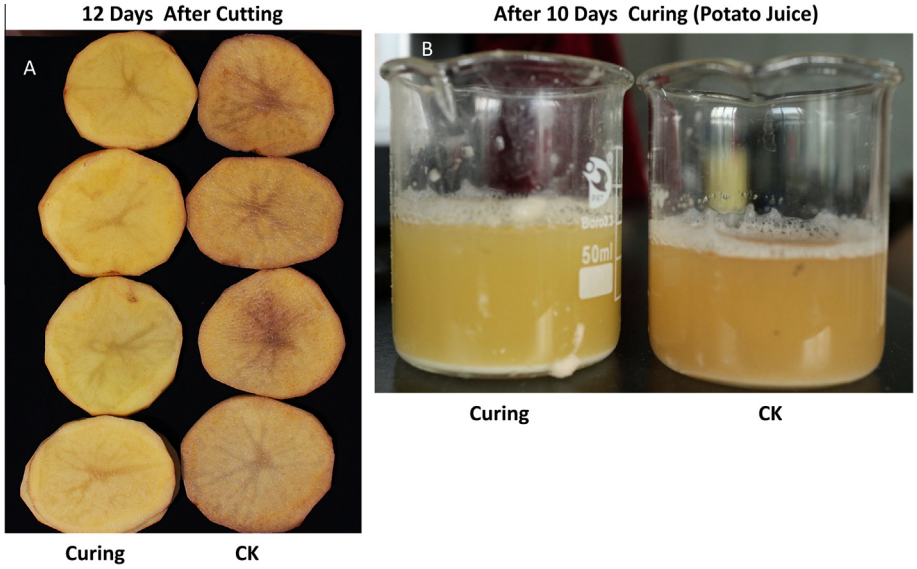

Fig. 3. The effect of curing temperature on potato flesh browning. (A) Potato fresh cut slices 12 d after cut, curing: potato stored at 16 °C for 10 d then used for fresh cut, CK: potato stored at 3 °C for 10 d then used for fresh cut. Picture was taken 12 d after cut. (B) Potato blended from the curing treated potato and control potato after the 10-d curing period, picture was taken 10 min after blend.

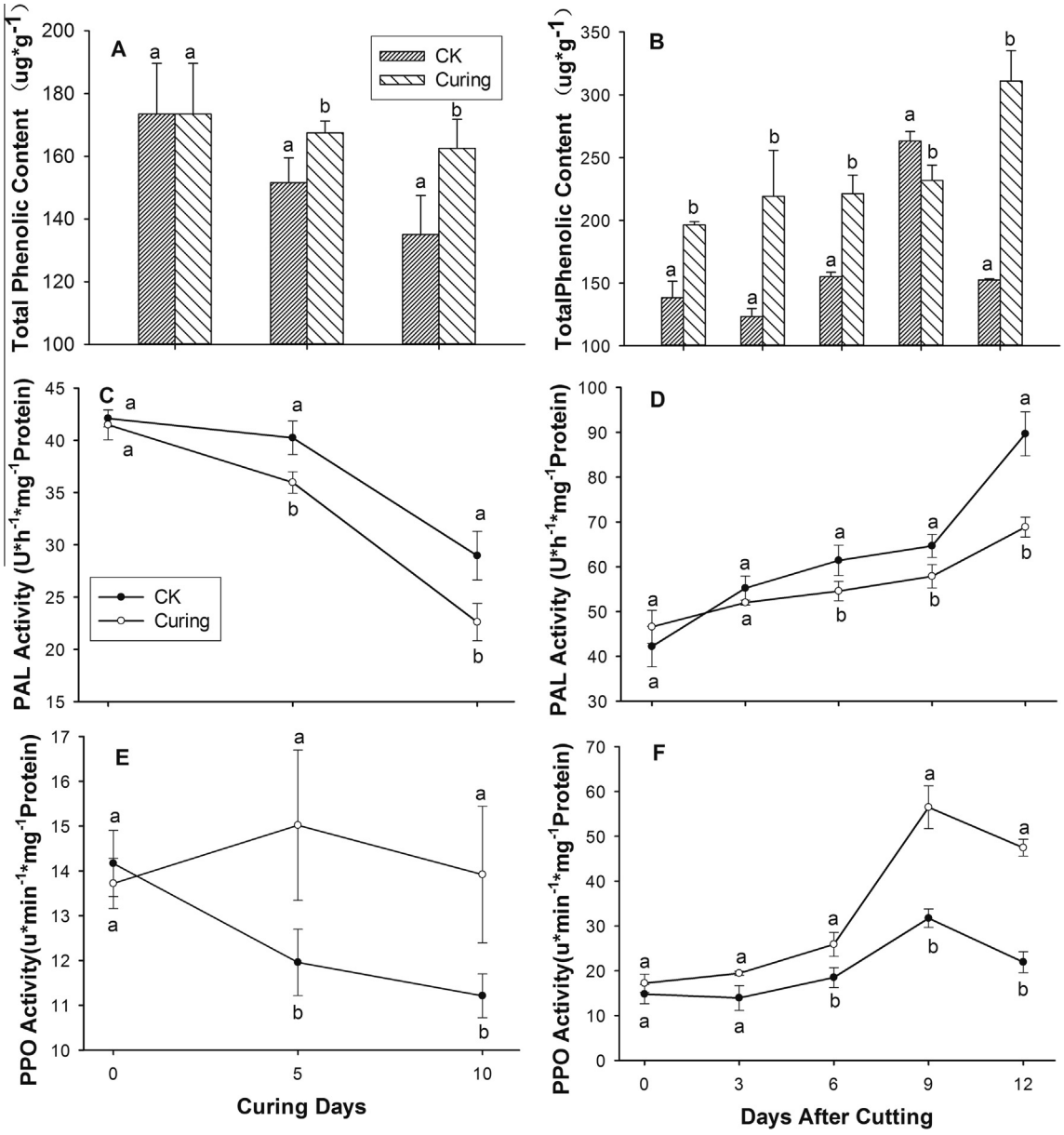

Fig. 4. (A–F) Effects of curing treatment on potato phenolic metabolism during the curing and after cutting. (A) Total phenolic content during curing, (B) total phenolic content after cutting, (C) PAL activity during curing, (D) PAL activity after cutting, (E) PPO activity during curing, (F) PPO activity on 0, 3, 6, 9, 12 d after cutting from the curing and control potato, respectively. Data are averages of three replications ± standard deviation. Test of the significance of difference is shown in the figure, "*a*" vs "*a*" meaning there is no significant difference ($P > 0.05$); "*a*" vs "*b*" meaning there is significant difference ($P \leqslant 0.05$).

associated with high phenolic compounds and less browning of romaine lettuce (Luna, Tudela, Martínez-Sánchez, Allende, & Gil, 2013). Mishra, Gautam, and Sharma (2013) reported that in eggplant, browning is dependent on soluble phenolic and PPO activity during storage. However, Cantos et al. (2002) found no relationship between PPO activity and browning in potato. Our data indicated that higher PPO activity does not always correlate with high browning compared with different treatment. Browning definitely involves phenolic oxidation, however, phenolic compound concentration could also be affected by many factors, such as synthesis, degradation, oxidation and transform, while enzyme activity could be affected by activator, inhibitor *in vivo*. Simple comparison of phenolic content, PPO, PAL activity in same time point is not enough to explain the relationship between browning and certain components; it needs a system acquiring and may have a time response gap. Compared with the control, cured potato slices acquired lower PAL activity and higher PPO activity and higher phenolic compound in the early stage of cutting, resulted in a lower browning of the fresh cut at the same time point with control sample. Our data demonstrated a temporal correlation between PPO activity and phenolic content in the same treatment but not compared with the different treatments. Phenolic compounds increases after wound (fresh cut) resulted in an increased PAL activity in both curing and control samples. However, higher total phenolic content and much higher chlorogenic acid, PPO activity and together with lower PAL activity on the day 12 contributed to the lower browning in the fresh cut slices from the curing treatment compared to the control.

3.5. Respiration rates and browning in juice

Respiration rates of the slices from the curing treated samples were lower than those of the control slices throughout 12 d shelf life period (Fig. 6). Higher than expected respiration rate was

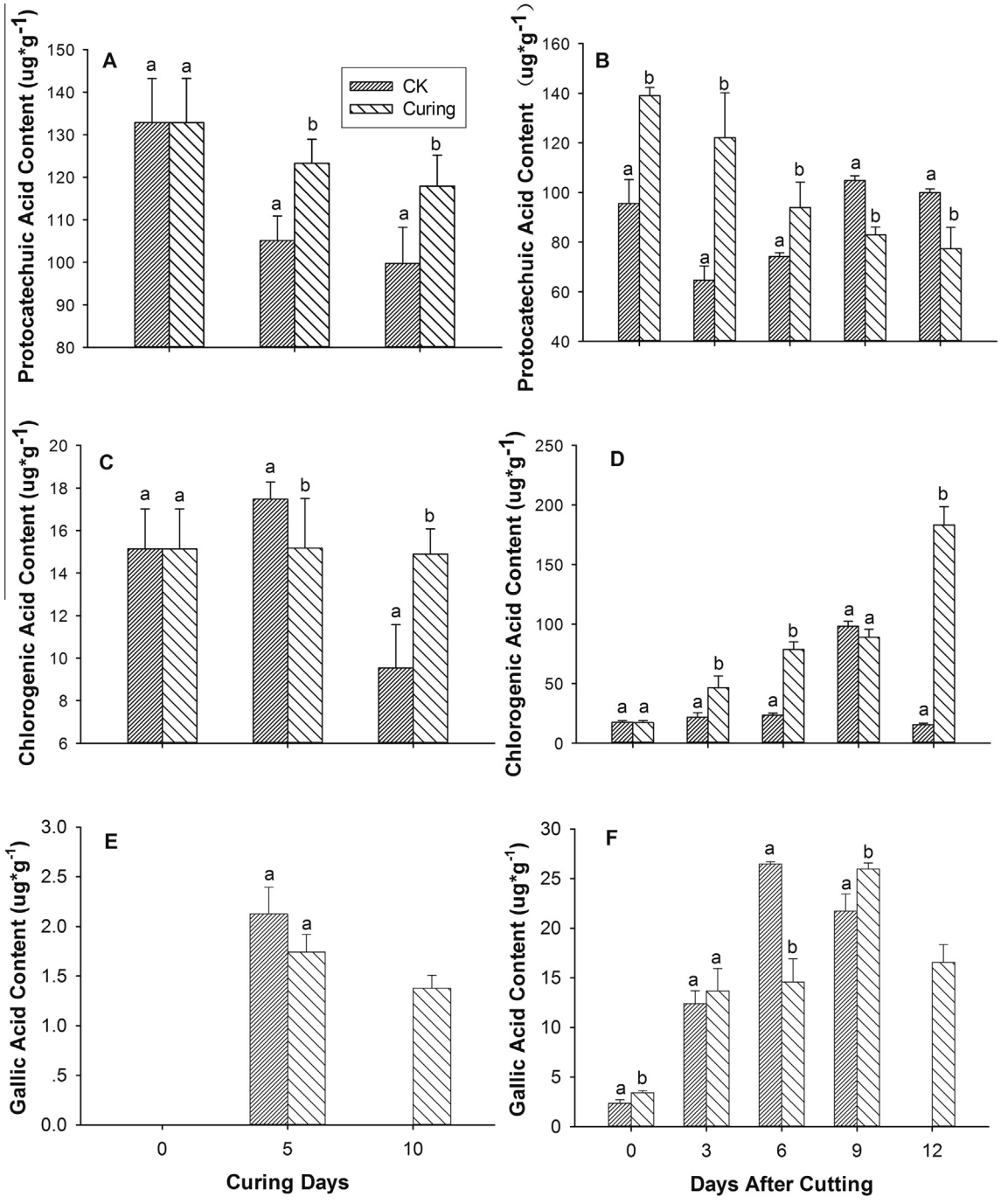

Fig. 5. (A–F) The individual phenolic compounds calculated by HPLC PDA absorbance peak area at the either 280 nm or 326 nm from the curing or after fresh-cut potato slices stored in the plastic bag at 3 °C. Data are the averages of three replications ± standard deviation. Left panel represents data from the day 0, 5, 10 during the curing, the right panel represents data from day 0, 3, 6, 9, 12, after cut from previously curing treated or control samples. (A and B) Protocatechuic acid at 280 nm with RT 11 min, matched with the standard in Supplemental Fig. 3; (C and D) chlorogenic acid at 326 nm with RT 22 min which is remarkable induced after fresh cut; (E and F) gallic acid at 280 nm with RT 5.4 min, limited peak height is detected during the curing before fresh cut (left); rapid increased after fresh cutting (right). Data are the averages of three replications ± standard deviation. Test of the significance of difference is shown in the figure, "*a*" vs "*a*" meaning there is no significant difference ($P > 0.05$); "*a*" vs "*b*" meaning there is significant difference ($P \leqslant 0.05$).

observed immediate after cut and 3 d after cut both in the curing treated and control slices because of the wounding response (Tapia et al., 2008). The results indicate that curing treatments decreased potato respiration rate of fresh-cut potatoes compared to control, which may be related to low metabolism in the tissue and low browning. Our results were consistent with the findings by Zhang, Zhan, Wang, and Tang (2008) in that the respiration rate was proportional to the extent of the browning. Higher respiration rates indicate a faster overall metabolism and deterioration (Chung & Moon, 2009).

The initiation of enzymatic browning is related to a loss of membrane integrity, due to the de-compartmenting of enzymes and substrates. The loss of membrane integrity was associated with the browning of fresh-cut potatoes (Jiang, Duan, Joyce, Zhang, & Li, 2004). However, in our experiment, the inhibition of browning in the curing sample is achieved even after blend to

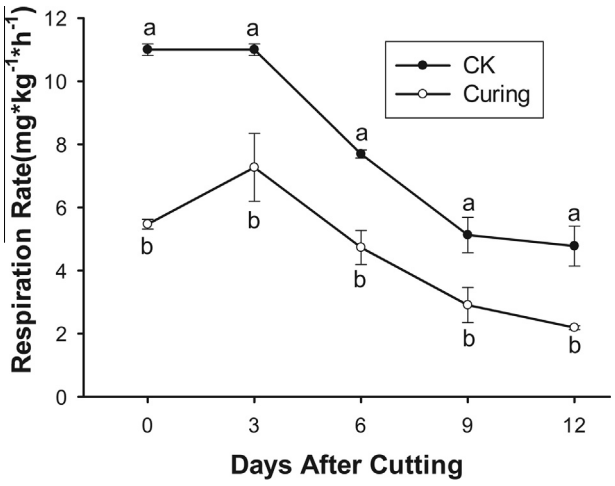

Fig. 6. The effect of postharvest curing on respiration rates of fresh-cut potatoes. Data are the averages of three replications ± standard deviation. Test of the significance of difference is shown in the figure, "*a*" vs "*a*" meaning there is no significant difference ($P > 0.05$); "*a*" vs "*b*" meaning there is significant difference ($P \leqslant 0.05$).

potato juice where the substrate and enzyme is mixed (Fig. 3B). The results imply that either higher antioxidant activity or less substrate, resulted from higher phenolic content during the curing, may serve as a main contributor for inhibiting browning in the fresh cut potato, while higher PPO activity in vitro may not indicate the high activity *in vivo*. In other words, the browning of potato fresh cut is not totally relied on PPO, especially compared with different treatment. Like other dietary polyphenols, chlorogenic acids are an excellent antioxidant. In vitro, it scavenges radicals generated in the aqueous phase, increases the resistance of LDL (Low Density Lipoproteins) to lipid peroxidation and inhibits DNA damage (Gordon & Wishart, 2010). *In vivo*, when ingested with the diet, caffeic acid and chlorogenic acid increase the plasma antioxidant capacity, the concentrations of endogenous antioxidants such as vitamin E and the *ex vivo* resistance of lipoproteins to oxidation. Therefore, the membrane integrity and resistance to lipoproteins oxidation by phenolic compounds such as chlorogenic acid together contributed to the less browning of flesh from the curing treated potato during storage. A systematic investigation of browning mechanism is required from gene expression, enzyme activity and metabolic from curing treatment and control. Global RNA sequence analysis using RNA-seq technology is underway to understand the molecular mechanism of potato flesh browning.

4. Conclusions

Curing treatment (10 d at 16 °C) immediately after harvest not only maintained higher phenolic content and PPO activity and lower PAL activity of potato tuber flesh, but also affected phenolic metabolism after fresh cut, and improved fresh cut colour, overall sensory quality and increased polyphenolic compound such as chlorogenic acid for 12 d after cut. PAL activity decreased in flesh of potato stored at both curing and control temperature before cut but increased in the 0–12 d after cut. Relatively higher PPO activity and phenolic compound concentration during the curing and after cut were associated with a lower browning in the curing tissue compared to the control. Protocatechuic acid and chlorogenic acid are predominated phenolic compounds in potato tuber flesh upon harvest, while the metabolisms of chlorogenic acid, gallic acid were found associated with increased PAL and PPO activities during 12 d storage period after cut and associated with the flesh anti-browning. The results demonstrate a simple, low cost method for preserving fresh-cut potatoes slices with the advantage of inhibited browning and increased antioxidant.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.08.011.

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