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RESEARCH ARTICLE

Combination of soy protein isolate and calcium chloride inhibits browning and maintains quality of fresh-cut peaches

Peng Han[1†], Bing-Hui Tang[2†], Chun-Ping Guo[1], Guang-Ling Shui[1], Zhen-Yuan Pan[1*], Hai-Rong Lin[1*]

[1]Key Laboratory of Oasis Ecology Agricultural of Xinjiang Production and Construction Corps, Agricultural College, Shihezi University, Shihezi, Xinjiang, China, [2]Cotton Research Institute of the Shihezi Academy of Agriculture Science, Shihezi, Xinjiang, China
[†]These authors have contributed equally to this work and share first authorship.

ABSTRACT

Fresh-cut fruits are often more perishable and exhibit browning shortly after pulp is cut and exposed to natural atmosphere. In this study, fresh-cut peaches were treated with distilled water (control), 20 g L^{-1} soy protein isolate (SPI), 10 g L^{-1} calcium chloride ($CaCl_2$) or 20 g L^{-1} soy protein isolate + 10 g L^{-1} calcium chloride (20 g L^{-1} SPI + 1.0 g L^{-1} $CaCl_2$), respectively, and then stored at 0°C to assess the protective effects of these treatments on the qualities and the antioxidative system of fresh-cut peaches. The result showed that firmness, colour (ΔE^*) and soluble solid content (SSC) in fresh-cut peaches treated with 20 g L^{-1} SPI + 10 g L^{-1} $CaCl_2$ were higher than control. Compared with other treatments, treatment with 20 g L^{-1} SPI + 10 g L^{-1} $CaCl_2$ significantly decreased weight loss rate, bacterial growth, respiratory rate and ethylene emission of fresh-cut peaches. Treatment with 20 g L^{-1} SPI + 10 g L^{-1} $CaCl_2$ also inhibited increase in browning degree, production of reactive oxygen species, malonaldehyde and total phenols, and maintained higher activities of superoxide dismutase and catalase, whereas inhibited the activities of peroxidase and polyphenol oxidase. These results suggest that treatment with 20 g L^{-1} SPI + 10 g L^{-1} $CaCl_2$ enhanced antioxidant activities of fresh-cut peaches, inhibited browning, and was more effective in maintaining the quality of fresh-cut peaches.

Keywords: Edible coating; Ethylene emission; Phenolic contents; Respiration rate; Active oxygen species

INTRODUCTION

Fresh-cut fruits, as healthy, nutritious, ready-to-serve products, are being highly demanded worldwide (Ansah et al. 2018). Browning, that limits the quality of fresh-cut fruits, includes enzymatic and non-enzymatic browning (Capotorto et al. 2018). The enzymatic browning of fruit and vegetables has been well reviewed recently (Singh et al. 2018). Phenols are substrates of respiration and precursors of melanin-quinone formation, so phenols are critical in determining browning intensity (de la Rosa et al. 2019). Polyphenol oxidase (PPO) is the primary enzyme for oxidation and catalyse enzymatic browning process. Peroxidase (POD) scavenge reactive oxygen species (ROS) like H_2O_2 which is a catalyst for oxidation of phenolic compounds and is considered deteriorative in flavour, texture, colour, and nutritional quality of foods (Singh et al. 2018). The inhibition of PPO and POD activities can reduce the degree of browning (Singh et al. 2018). Superoxide dismutase (SOD) dismutates O_2^- into O_2 and H_2O_2 whereas catalase (CAT), in peroxisome, dismutase's H_2O_2 into water and oxygen (Racchi 2013).

Peach (*Amygdalus persica* L.) is one of the famous distinctive fruits in Xinjiang, China. Moreover, fresh-cut peaches occupy an important role in the fresh-cut market (Wu et al. 2022). Thus, it is a challenge to improve the preservation technologies for the fresh-cut peaches industry.

There are physical, chemical, and biological methods to preserve fresh-cut peaches. Physical methods include exposure of food to low temperature(Meng et al. 2009; Zhang et al. 2022b), heat (Steiner et al. 2006), light (McDonald et al. 2012), and modified atmosphere

***Corresponding author:**
Zhen-Yuan Pan, Agricultural College, Shihezi University, 832003, Xinjiang, China. Email: panzhenyuandawood@163.com.
Hai-Rong Lin, Agricultural College, Shihezi University, 832003, Xinjiang, China. Email: 1219491670@qq.com.

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packaging (Malakou et al. 2005; Özkaya et al. 2016). Acidic electrolyzed water (Zhi et al. 2017), nanotechnology (Liu et al. 2020; Zhang et al. 2022a) and bio-preservative methods (Islam et al. 2018) are also widely used in fresh-cut preservation. Some chemicals are also commonly used as anti-browning agents in the food preservation (Oms-Oliu et al. 2010) which include hydrogen sulfide (Wang et al. 2023), ascorbate calcium (Sortino et al. 2022), 1-methylcyclopropene and phytic acid (Jiang et al. 2023) and L-cysteine (Gohari et al. 2021). Plant-based edible coatings are also commonly used to manage postharvest quality and extend shelf-life of fresh-cut peaches (Ayala-Zavala et al. 2013).

Soy protein isolate (SPI) is a newly developed material in the preservation of fresh-cut products. SPI has good emulsification, gelling and foaming properties, can reduce surface tension between water, air and product, and forms a film having resistance to damage (Tian et al. 2018; Yousuf et al. 2018). SPI is usually used together with other bacteriostatic agents to form complexes at the nanoscale to preserve fruits through hydrophobic interactions (Alves et al. 2017). SPI together with other reagents can inhibit browning of fresh-cut apples (Alves et al. 2017) as it has been reported to exhibit antibacterial and antioxidant properties (Liang et al. 2018). Calcium chloride ($CaCl_2$) protects adhesion layer structure of cells, reduce cell wall decomposition, stabilize the membrane structure, and extend the shelf life of fruits. $CaCl_2$ also inhibits reaction of phenolic acid with oxygen thereby presents anti-browning characteristics (Jiao et al. 2017) {Mansourbahmani, 2017 #48; Wenxiao Jiao, 2018 #14}. Also, $CaCl_2$ has excellent antibacterial properties and can maintain the quality of fresh-cut fruits (Ayón-Reyna et al. 2015). Currently, $CaCl_2$ has been used in conjunction with coating agents or with modified atmospheres (Ghidelli et al. 2015). However, $CaCl_2$ is slightly bitter in taste and is extremely hygroscopic. With the good emulsifying properties and film-forming properties, SPI may also be used with $CaCl_2$ as a preservative. However, there are few reports on the preservation ability of fresh-cut fruits by the combination of SPI and $CaCl_2$, especially on peach.

To investigate techniques for maintaining the freshness of fresh-cut peaches, fresh-cut peach slices were immersed in SPI, $CaCl_2$ or a mixture of the two for five minutes. After air drying under shade, the hardness, color and soluble solid content were evaluated at 0, 2, 4, 6, 8 and 10 days to evaluate the quality of fresh-cut peaches. Explore the effect of different methods on the quality preservation of fresh-cut peaches, and provide a new and effective method for maintaining the quality of fresh-cut peaches.

MATERIALS AND METHODS

Plant materials

Peaches were harvested from a local orchard (Shihezi, China), and grouped into four lots (50 fruits per lot), then pre-washed in 0.5 ml L^{-1} chlorinated water for 3 min to reduce natural microflora. Fruits were washed with sterile water to remove chlorine residues. Peaches were then cut into 2 cm thick slices. The results of the preliminary studies showed that both 20 g L^{-1} SPI and 10 g L^{-1} $CaCl_2$ maintain quality of fresh-cut peaches. Therefore, fresh-cut peaches were dipped in distilled water (as a control treatment), 20 g L^{-1} SPI, 10 g L^{-1} $CaCl_2$ or 20 g L^{-1} SPI+10 g L^{-1} $CaCl_2$ for 5 min. After air drying under shade, all fresh-cut peaches were stored at 0 °C.

Determination of colour, firmness, soluble solid content, and browning degree

A colorimeter (CR-10 Konica Minolta, Japan) was used to detect the colour (Orikasa et al. 2017). L^* (whiteness or light/dark), a^* (red/green) and b^* (yellow/blue) and express changes in surface colour of fresh-cut peach samples. The total colour difference (ΔE^*) was calculated using formula (1).

$$\Delta E^* = \sqrt[2]{\left(L^*\right)^2 + \left(a^*\right)^2 + \left(b^*\right)^2} \quad (1)$$

The firmness of fresh-cut peaches was measured by performing a pressure test with 5 mm cylindrical probe using a CY-4 fruit firmness tester (Shanghai Hill Company) and expressed as N cm^{-2}.

SSC was measured with refractometer (WYT-15, Shanghai Cany Precision Instrument Co. Ltd, Shanghai, China), and the unit was represented by Brix.

As Lei et al. (Lei et al. 2018) described, the browning degree (BD) of fresh-cut peaches was estimated at 410 nm by a spectrophotometer (Shimadzu, Japan).

Determination of weight loss, respiratory rate, ethylene content and total number of colonies

The weight loss rate was calculated using equation (weight before storage - weight at sampling time)/weight before storage.

Respiratory rate was measured by Fruit and Vegetable Respiratory Measuring Instrument (SY-1022, Shijiazhuang Shiya Technology Co., Ltd.) and averaged three times (mg kg^{-1} h^{-1}).

The method of Guerreiro et al. (2017) was carried out to estimate amount of ethylene released(μL kg^{-1} h^{-1}).

The total number of colonies was measured according to Khandpur et al. (2016) and expressed in colony-forming units (CFU g^{-1} FW).

Determination of the contents of ROS, malonaldehyde and total phenols

The method of Jambunathan (2010) was executed to estimate ROS content. ROS was determined with a fluorescence spectrophotometer at maximum excitation and emission wavelengths of 485 nm and 530 nm, respectively.

As the method of Liu et al. (2018) depicted, malonaldehyde (MDA) content was acquired at the absorbance (OD) of 450 nm, 532 nm and 600 nm. MDA content was calculated by taking advantage of the following formula (2) and expressed as mmol g^{-1} (mmol g^{-1} FW) of fresh weight basis.

$$MDA = 36 \times \begin{bmatrix} 6.45 \times (OD_{532} - OD_{600}) \\ -16 \times OD_{450} \end{bmatrix}$$

The Folin-Ciocaulten method was applied to assay total phenol content under the absorbance at 765 nm (Koley et al. 2016).

Determination of the activities of PPO, SOD, CAT and POD

For the PPO, SOD, and CAT activity, the supernatant was prepared following procedure of Zhu et al. (2008). PPO activity was estimated as earlier described by Lin et al. (2016). Ali et al. (2018) defined one unit (U) of SOD activity as inhibition of the photoreduction of nitro-blue tetrazolium (NBT) by 0.5. The CAT activity was assayed by estimating the decrease in absorbance due to reaction of H_2O_2 at 240 nm for 120 s according to the method of Li et al. (2017). The method of Zhu et al. (2008) was carried out to estimate POD activity at the absorbance of 580 nm. The activity of these enzymes was expressed as U g^{-1} protein min^{-1}.

Statistical analysis

All experiments were performed in completely randomized design with three replicates per sample, and the data were analysed by one-way analysis of variance (ANOVA). Differences were significant at *P*-value less than or equal to 0.05. All data were expressed as mean ± standard error (n = 3).

RESULTS

Changes in total colour difference, browning degree, firmness and soluble solid contents

Total colour difference (ΔE^*) value of fresh-cut peaches continued to increase during storage (Fig. 1A). Both treatments with SPI and $CaCl_2$ delayed augment of total colour difference. However, total colour difference value in fresh-cut peaches treated with SPI+$CaCl_2$ was markedly lower than that of other treatments. Total colour difference value of peaches treated with SPI+$CaCl_2$ was 4.48, which was about 2.61, 1.98, 1.35-fold smaller than that of the control, SPI, $CaCl_2$, respectively, at day 10. The results from total colour difference analysis suggested that treatment of SPI+$CaCl_2$ effectively delayed browning of fresh-cut peaches. Browning degree of fresh-cut peaches increased during the storage (Fig. 1B). Both treatments with SPI and $CaCl_2$ significantly inhibited increase in browning degree of fresh-cut peaches during storage. However, difference was non-significant in browning degree between SPI and $CaCl_2$ treatments. Treatment with SPI+$CaCl_2$ maintained lowest browning degree during storage when compared with other treatments. The firmness of fresh-cut peaches decreased quickly during storage (Fig. 1C). Especially on day 10, the firmness of the control was 1.7-fold lower than firmness determined before storage. Treatment with SPI+$CaCl_2$ delayed decrease of firmness in fresh-cut peaches. Therefore, firmness of fresh-cut peaches treated with SPI+$CaCl_2$ was significantly better than control after day 4. Especially on day 10, the firmness of fresh-cut peaches treated with SPI+$CaCl_2$ was 1.28-fold higher than other treatments including control. However, before day 8, no significant difference between SPI-treated peach slices and control was found with respect to firmness. Analogously, firmness of fresh-cut peaches treated with $CaCl_2$ was also higher than control.

SSC of fresh-cut peaches declined during storage (Fig. 1D). There was a non-significant difference in SSC among fresh-cut peach slices treated with either $CaCl_2$, SPI+$CaCl_2$ or control. However, treatment with SPI dramatically inhibited the decline in SSC of fresh-cut peaches during storage. It was apparent that SSC in fresh-cut peach slices treated with SPI remained higher than control in fresh-cut peaches during storage.

Changes in weight loss, microbial growth, ethylene production and respiratory rate

Rate of weight loss increased with advancement in storage period (Fig. 2A). Increase in weight loss was also reflected in loss of firmness. Treatment of fresh-cut peach slices with SPI, $CaCl_2$ or SPI+$CaCl_2$ significantly hindered increase in weight loss in fresh-cut peaches compared to control. Weight loss in untreated fresh-cut peaches (control) was 1.27 and 1.12-fold higher than SPI+$CaCl_2$-treated peach slices on day 6 and 10, respectively.

Fresh-cut fruits are more susceptible to microbial growth because of large amount of water and sugar on their surface. The results showed that microbial growth on fresh-cut peaches treated with SPI+$CaCl_2$ was consistently lower than control (Fig. 2B). This bacteriostatic effect lasted until the last day of storage. Compared to SPI and $CaCl_2$ treatments, treatment with SPI+$CaCl_2$ significantly inhibited growth of microorganisms. Fresh-cut peaches treated with

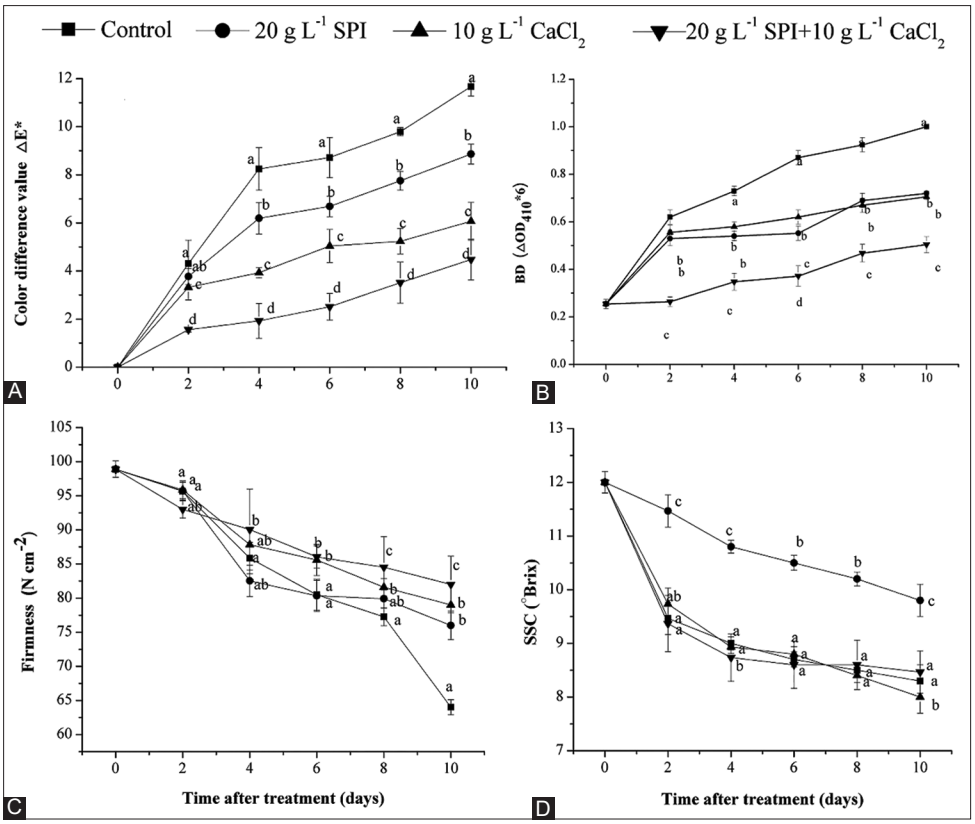

Fig 1. (A-D) Effects of soy protein isolate and calcium chloride on colour difference value, browning degree, firmness and soluble solid contents in fresh-cut peach slices. Means followed by a different letter on same day are significantly different (P <0.05). Note: BD, browning degree; SPI, soy protein isolate; SSC, soluble solid contents.

SPI+$CaCl_2$ also exhibited delay in ethylene production and rate of respiration (Fig. 2C, D). At day 10, ethylene produced from control samples was 1.25-fold higher than SPI+$CaCl_2$-treated samples. On 8th day, production of CO_2 from untreated peach slices was 29.05 mg kg^{-1} h^{-1}, which was 1.65-fold higher than SPI+$CaCl_2$.

Changes in ROS, MDA and total phenolic content

All treatments reduced accumulation of ROS, MDA and total phenolic content in fresh-cut peaches (Fig. 3A, B). Contents of ROS and MDA in fresh-cut peaches treated with SPI+$CaCl_2$ were significantly lower than other treatments including control. ROS contents in SPI-treated and $CaCl_2$-treated fresh-cut peaches were non-significantly different. On day 6, MDA content in fresh-cut peaches treated with SPI whereas MDA content in fresh-cut peaches treated with $CaCl_2$ were lower than control. Phenolic contents in peach slices treated with SPI, $CaCl_2$ or SPI+$CaCl_2$ were significantly lower than control but non-significantly different than each other during storage (Fig. 3C).

Changes in polyphenol oxidase, peroxidase, superoxide dismutase and catalase activities

The PPO and POD activities of fresh-cut peaches in all treatments were significantly lower than control (Fig. 4A, B). There was no difference in the level of PPO activity between treatments with SPI or $CaCl_2$ during storage. However, POD activity of fresh-cut peaches treated with $CaCl_2$ was higher at day 2 and lower after day 4 than in peaches treated with SPI. PPO and POD activities of fresh-cut peaches treated with SPI+$CaCl_2$ were significantly lower than in peaches treated with SPI and $CaCl_2$ alone.

Compared to the control, all treatments delayed decrease in activities of SOD and CAT in fresh-cut peaches during storage (Fig. 4C, D). However, the activities of SOD and CAT in peaches treated with SPI+$CaCl_2$ were significantly higher than in peaches treated with $CaCl_2$ or SPI alone. Similarly, SOD activity in $CaCl_2$-treated peaches was higher than SPI-treated peaches. However, CAT activity in $CaCl_2$ or SPI-treated fresh-cut peaches remained non-significantly different during storage.

DISCUSSION

Fresh-cut peaches are vulnerable to water loss, browning and contamination, and quickly lose their quality during storage. Both SPI and $CaCl_2$ reduce water loss, oxidative damage and inhibit browning, and maintain quality of

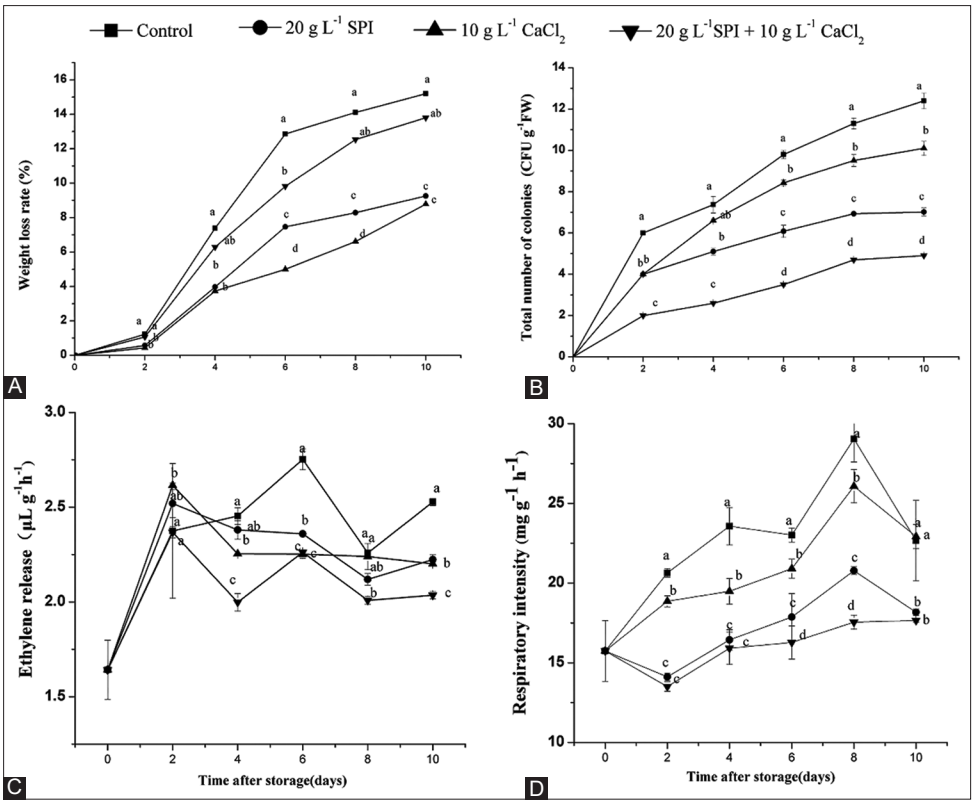

Fig 2. (A-D) Effects of soy protein isolate and calcium chloride on weight loss, microbial growth (total number of colonies), ethylene production and respiration rate in fresh-cut peach slices. Means followed by a different letter on same day are significantly different (P <0.05). CFU, colony forming unit; SPI, soy protein isolate.

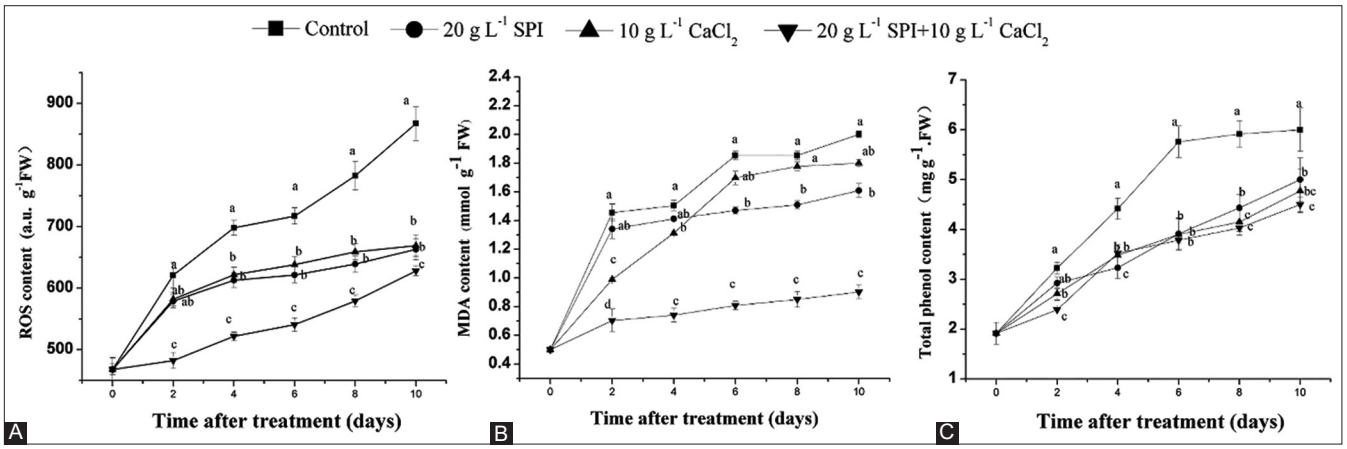

Fig 3. (A-C) Effects of soy protein isolate and calcium chloride on reactive oxygen species content, malonaldehyde content and total phenol content in fresh-cut peach slices. Means followed by a different letter on same day are significantly different (P <0.05). MDA, malonaldehyde; ROS, reactive oxygen species; SPI, soy protein isolate.

fresh-cut peaches during storage. The combination of SPI and $CaCl_2$ exhibited more positive impacts on reducing browning and retaining quality of fresh-cut peaches than treatment with either SPI or $CaCl_2$ alone.

In general, many changes in physicochemical attributes affect produce quality and can be used as indicators of consumer satisfaction with the product. These attributes include difference in colour change, firmness, and SSC. Colour is an essential vital indicator of browning, but also the primary factor in determining willingness of consumers to purchase. The results showed that the fresh-cut peaches treated with 20 g L^{-1} SPI+10 g L^{-1} $CaCl_2$ had least change in colour difference value (Fig. 1A), which was similar to the results of the previous reports (Ghidelli et al. 2015; Qi et al. 2011). SPI has high viscosity, plasticity, and elasticity, and can be used as a carrier for water, as well as a preservative for flavour, sugars and other quality attributes (Alves

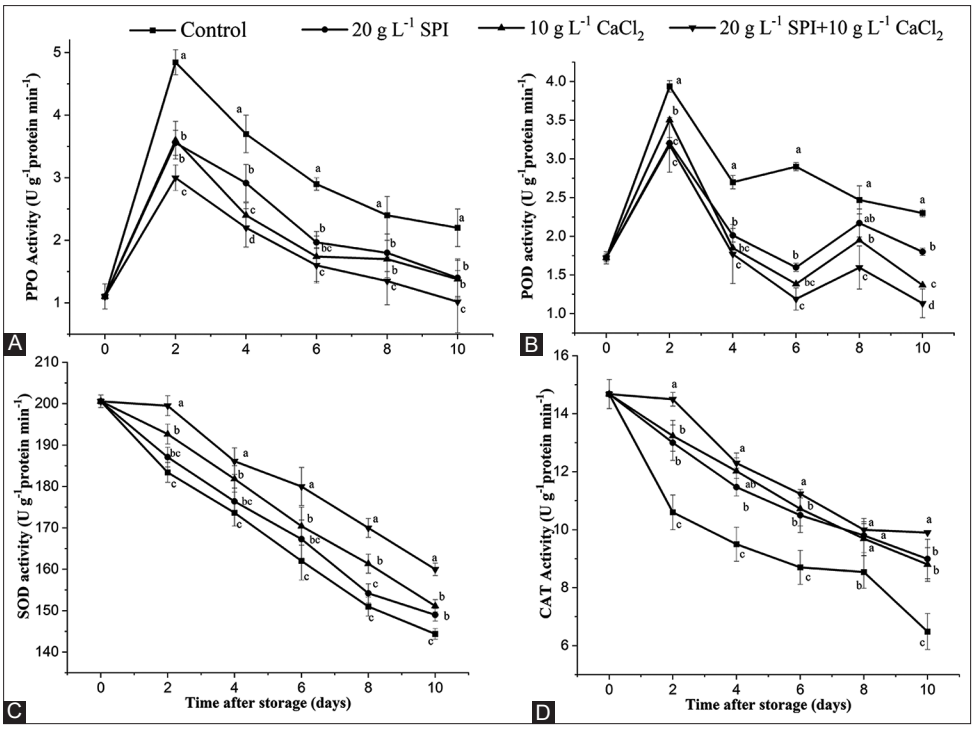

Fig 4. (A-D) Effects of soy protein isolate and calcium chloride on polyphenol oxidase, peroxidase, superoxide dismutase and catalase activities in fresh-cut peach slices. CAT, catalase; POD, peroxidase; PPO, polyphenol oxidase; SOD, superoxide dismutase; SPI, soy protein isolate.

et al. 2017). So, the fresh-cut peaches treated with 20 g L^{-1} SPI+10 g L^{-1} $CaCl_2$ had a higher value of SSC than peaches treated with 10 g L^{-1} $CaCl_2$ (Fig. 1D). SPI coating on fresh-cut peaches may also have possibly decreased water loss and oxygen permeability, which reduced the browning of peaches (Fig. 1B). The firmness is one of the attributes that dictates the postharvest life (Chen et al. 2015). Treatment of 20 g L^{-1} SPI + 10 g L^{-1} $CaCl_2$ maintained firmness of fresh-cut peaches followed by 10 g L^{-1} $CaCl_2$ (Fig. 1C). These results suggested that $CaCl_2$ plays an essential role in maintaining the firmness of fresh-cut fruit. $CaCl_2$ maintains pectinase activity, and Ca^{2+} form connecting bridges between the free carboxyl groups in the pectin chain which leads to cell wall enhancement and tissue tightening thereby maintaining its firmness (Chong et al. 2015).

During storage, ROS, such as O_2^-, H_2O_2, and -OH, are continuously generated (Gutteridge et al. 2018). Excessive ROS accelerates the oxidative damage of fruits and affects quality of the produce (Chen et al. 2018). MDA plays a vital role in membrane lipid peroxidation, which can intensify membrane damage. In this study, amount of ROS and MDA in fresh-cut peaches increased during storage and were significantly decreased by the treatments with SPI and $CaCl_2$ (Fig. 3A, B), suggesting that SPI and $CaCl_2$, especially the combination of SPI and $CaCl_2$, enhanced capacity of fresh-cut peaches to resist the oxidative stress. It is plausible that $CaCl_2$ interacted with polar groups of the SPI through the calcium bridge, which enhanced the gelation of SPI (Tian et al. 2018), and help SPI to be more stable to form a protective film around tissue surface and protect cells. The SPI film may have hindered permeation of atmospheric oxygen to fresh-cut surface which lead to decrease in non-enzymatic browning of fresh-cut peaches. The oxidation of phenolic compounds into quinones is catalysed by PPO and POD which then react with tannins to form brown polymers that negatively affect fruit quality (Can-Cauich et al. 2017). Treatment with 20 g L^{-1} SPI+10 g L^{-1} $CaCl_2$ delayed changes in PPO and POD activities (Fig. 4A, B), which decreased the enzymatic browning and led to a low browning degree of fresh-cut peaches (Fig. 1B). Similar results are also reported by Jiao et al. (Jiao et al. 2017).

SOD and CAT are important antioxidative enzymes. The activities of SOD and CAT measured in this experiment showed a decreasing trend as earlier reported by Koushesh Saba and Sogvar (Koushesh Saba et al. 2016) and Gao et al. (Gao et al. 2018). Treatment with 20 g L^{-1} SPI+10 g L^{-1} $CaCl_2$ inhibited decrease in activities of SOD and CAT (Fig. 4C, D), which resulted in a lower generation of ROS of fresh-cut peaches during storage (Fig. 3A). These results suggest that treating fresh-cut peaches with 2% SPI+1% $CaCl_2$ reduced oxidative damage caused by ROS, which was helpful to maintain quality of fresh-cut peaches during storage.

SPI contains many polar groups along with its peptide chain backbone, which has water retention properties and regulates enzymatic activities in fresh-cut peaches

favouring quality preservation (Ghidelli et al. 2018). $CaCl_2$ is also used as a calcium fortifier, chelating agent, desiccant, microbicide, and tissue modifier in the food industry (Kubo et al. 2018). Treatment with 20 g L^{-1} SPI+10 g L^{-1} $CaCl_2$ exhibited synergistic effects of SPI and $CaCl_2$ on delaying browning and maintaining quality of fresh-cut peaches. However, further studies are needed to demonstrate effectiveness of SPI+$CaCl_2$ treatment to inhibit browning from a molecular perspective. It is also needed to investigate that whether SPI reacts with $CaCl_2$ to form new structures to play positive roles.

CONCLUSIONS

Treatment with 20 g L^{-1} SPI+10 g L^{-1} $CaCl_2$ significantly delayed browning and maintained quality of fresh-cut peaches. Oxidative damage caused by ROS in fresh-cut peaches was reduced by treating peach slices with 20 g L^{-1} SPI+10 g L^{-1} $CaCl_2$. The synergistic effect of 20 g L^{-1} SPI+10 g L^{-1} $CaCl_2$ on preserving quality of peach slices was more pronounced than SPI or $CaCl_2$ application alone.

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Author Contributions

Zhen-Yuan Pan, and Hai-Rong Lin conceived and designed the experiments. Guang-Ling Shui, Chun-Ping Guo and Bing-Hui Tang conducted preliminary experiments and determined the experimental concentration. Bing-Hui Tang and Peng Han collected and analyzed the data. Bing-Hui Tang and Peng Han wrote the manuscript. Zhen-Yuan Pan, and Hai-Rong Lin revised and reviewed the article.

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