Food Chemistry: Molecular Sciences 9 (2024) 100229

Contents lists available at ScienceDirect

Food Chemistry: Molecular Sciences

journal homepage: www.sciencedirect.com/journal/food-chemistry-molecular-sciences

Calcium chloride connects potato greening and enzymatic browning through salicylic acid

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ARTICLE INFO

Keywords:
Potato greening
$CaCl_2$
SA
Enzymatic browning

ABSTRACT

Greening and enzymatic browning are important factors causing post-harvest losses in potatoes. Although they are two different biological processes, there are some common inhibitors between them. Whether there is a correlation between the two has yet to be studies. In this research, we conducted transcriptome analysis of non-greening and greening potatoes, identifying several browning-related genes (polyphenol oxidase genes and peroxidase genes). Compared to non-greening potatoes, greening potatoes exhibited a greater browning degree. And calcium chloride ($CaCl_2$) can inhibit both greening and enzymatic browning. However, the inhibitory effect on potatoes was weakened when treated simultaneously with SA synthesis inhibitor and $CaCl_2$, indicating that $CaCl_2$ can regulate potato greening and browning by affecting internal SA synthesis. Additionally, exogenous SA treatment of potato tubers can also inhibit enzymatic browning. Our study not only demonstrated that $CaCl_2$ and SA can serve as a bridge connecting the potato greening and enzymatic browning, but also provided important references for the development of novel co-inhibitors.

1. Introduction

During the harvesting, storage, transportation, and sales processes of potato tubers (*Solanum tuberosum L.*), exposure to light causes the amyloplast in the tubers transform into chloroplasts (Kim et al., 2017; Tanios et al., 2020). When mechanically damaged, the phenolic substances in the tubers are oxidized by oxygen and polyphenol oxidase (PPO) into colored quinone compounds, leading to enzymatic browning. Both greening and enzymatic browning can lead to nutritional loss and a decrease in commodity value of potato tubers. Potato greening and browning are two different biological processes: one is a developmental process, while the other is a stress response process. However, some inhibitors that have been discovered can suppress both greening and browning. Ethanol fumigation can delay potato greening (Dong et al., 2017; Tang et al., 2023). Additionally, ethanol fumigation can inhibit the enzymatic browning of fresh-cut yams by suppressing the activity of phenylalanine ammonia-lyase, delaying the accumulation of total phenols, and reducing the production of malondialdehyde (Fan et al., 2018). Moreover, vacuum infiltration with 1 % or 2 % calcium chloride ($CaCl_2$) can inhibit chlorophyll synthesis and delay greening in '*Katahdin*' potatoes (*Solanum tuberosum L.*) (Arteca, 1982). And treatment with 1 % $CaCl_2$ can enhance the antioxidant capacity, inhibit PPO activity, thereby reducing the degree of browning in fresh-cut *Luffa cylindrica* (Feng, Feng, et al., 2022; Feng, Sun, et al., 2022). $CaCl_2$ treatment has been shown to result in lower levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) accumulation in loofah. (Feng, Feng, et al., 2022; Feng, Sun, et al., 2022). Additionally, vacuum impregnation of fresh-cut potatoes with ascorbic acid and calcium ascorbate for 2 min significantly reduced browning (Zhao et al., 2022). Whether there is a relationship between potato greening and browning, and whether there exists a signaling molecule that regulates both greening and browning, all these needs further research.

Salicylic acid (SA), as an important defense phytohormone, enhances plant resistance to biotic and abiotic stresses by activating the expression of disease resistance genes (pathogenesis-related gene, *PR*) and modulating the antioxidative enzyme system (superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD)) (Song et al., 2023). Fruits and vegetables exposed to abiotic stress such as mechanical injury can lead to the occurrence of enzymatic browning. SA treatment significantly increased the antioxidative enzyme activities of SOD, CAT, and

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https://doi.org/10.1016/j.fochms.2024.100229
Received 10 September 2024; Received in revised form 25 October 2024; Accepted 16 November 2024
Available online 22 November 2024

ascorbic acid peroxidase (APX) in morels (*Morchella sextelata*), enhanced their antioxidant capacity, reduced membrane lipid peroxidation, and inhibited enzymatic browning (Gao et al., 2022). SA can also act as a competitive inhibitor of PPO, reducing the degree of enzymatic browning in shelled and sliced chestnuts (Zhou et al., 2015). Moreover, treatment of tomato leaves with high concentration of SA (1 mM) causes damage to the chloroplast structure, with abnormal shape of plastids and increased thylakoid volume (Poór et al., 2019). It is still unknown whether SA signals regulate enzymatic browning and greening in potatoes simultaneously. And studies have shown that calcium signaling regulated the SA accumulation during immunity (Seyfferth & Tsuda, 2014). Whether calcium signals can regulate potato enzymatic browning and greening by affecting SA signals also requires further research.

Here, we report that $CaCl_2$ can regulate postharvest potato greening by affecting the internal SA content. And compared to non-greening potatoes, greening potatoes exhibit more severe browning after freshly cut. Interestingly, $CaCl_2$ can also inhibit enzymatic browning by affecting the internal SA content in potatoes. Then we demonstrated that SA can alleviate browning by enhancing antioxidant capacity, inhibiting PPO activity, and reducing quinone levels. Our study has revealed for the first time the correlation between potato greening and enzymatic browning, providing crucial insights for the development of novel inhibitors targeting greening and browning.

2. Materials and methods

2.1. Materials

Potatoes (cultivar, '*Netherlands 15*') were purchased from Yangzhuang Town, Laicheng District, Laiwu City, Shandong Province and then harvested in May 2023 and stored in 4 °C for 3 months. Selected uniformly size potato tubers weighing between 150–250 g, free from mechanical damage, deformities, sprouting, and greening for experiments. All chemicals used were of analytical or chemical grade and were purchased from Tianjin Kaitong chemical reagent Co., Ltd. (Tianjin, China) and Shanghai Macklin biochemical technology Co., Ltd. (Shanghai, China).

2.2. Screening the optimal soaking concentration for $CaCl_2$ solution

Potatoes were washed with clean water, soaked in a 200 mg L^{-1} NaClO solution for 3 min, and then dried with gauze. Then these potatoes were soaked in $CaCl_2$ solution with different concentrations (0.2 %, 0.4 %, 0.6 %, 0.8 %, and 1.0 %) for 10 min, and the untreated potatoes (soak in water for 10 min) were used as the control group. Then, the potato tubers were placed under fluorescence irradiation at 20 °C, 80 % relative humidity, and 25 W 24 h / d in a light culture chamber (MIR-254-PC., Osaka, Japan) to observe the degree of greening of the potato tubers. Finally, the optimal soaking concentration of $CaCl_2$ (0.8 %) was selected. Sixty potato tubers were used for screening the optimal soaking concentration of $CaCl_2$. Measurements were taken every 3 d for a total 12 d, with three repetitions each time.

2.3. Screening the optimal soaking time for $CaCl_2$

Potatoes were placed in 0.8 % $CaCl_2$ solution and soaked for 0 min, 5 min, 10 min, 15 min, and 20 min, respectively. The potatoes soaked for 0 min served as the control group. Then, the greening degree of potato tuber was observed under 20 °C, 80 % relative humidity, and fluorescence irradiation of 25 W 24 h / d. Finally, the optimal soaking time of $CaCl_2$ (20 min) was selected. Sixty potato tubers were used for screening the optimal soaking time of $CaCl_2$. Measurements were taken every 3 d for a total 12 d, with three repetitions each time.

Potato tubers were washed and immersed in 0.8 % $CaCl_2$ and 0.8 % $CaCl_2$ + 2 mM SA synthesis inhibitor (2-amino amino acid-2-phosphonic acid, AIP) for 20 min, with deionized water as a control, and wiped dry the surface water, with three repetitions each time. The outer skin of the potato was gently scraped with the handle of a medicine spoon and photographed for observation.

2.4. RNA extraction and quantitative RT-PCR analysis

Total RNA from the potato epidermis was extracted and cDNA was synthesized using the Whole Plant RNA Kit (DNaseI) and the HiFiScript cDNA Synthesis Kit (CWBIO Inc., Beijing, China). Primer pairs were designed using Primer Premier 5.0 and the sequences are described in Supplementary Table1. Gene expression levels were determined by quantitative real time-PCR (qRT-PCR) using the Power UltraSYBR mixing kit (CWBIO Inc., Beijing, China), and measured using the CFX 96 ™ real-time quantification system (Bio-Rad). The data obtained by qRT-PCR were normalized and calculated according to the $2^{-\Delta\Delta Ct}$ method (Upadhyaya et al., 2009).

2.5. Determination the greening of potato epidermis

According to the selected optimal treatment conditions, the potatoes were soaked with 0.8 % $CaCl_2$ solution for 20 min. The untreated potatoes were used as the control group. After the soaking, they were air-dried, and then placed in a light culture chamber (MIR-254-PC., Osaka, Japan) at 20 °C, 80 % relative humidity, and fluorescence irradiation of 25 W 24 h / d. Five potatoes were randomly selected for determination of greening degree. Four points were chosen on the top, bottom, left, and right of each potato surface. A 2 mm thick epidermis was cut from these points. Then, the a^* (red and green) values of the cut epidermis were determined using a colorimeter CR-400 (Minolta Co., Osaka, Japan).

2.6. Measurement of chlorophyll content

According to the selected optimal treatment conditions, the potatoes were soaked with 0.8 % $CaCl_2$ solution for 20 min. The untreated potatoes were used as the control group. After the soaking, they were air-dried, and then placed in a light culture chamber (MIR-254-PC., Osaka, Japan) at 20 °C, 80 % relative humidity, and fluorescence irradiation of 25 W and 24 h d^{-1}. Samples were taken every 3 d for a total 12 d. These samples were ground into powder by liquid nitrogen grinder (FW100 Taiste Instrument Co., Ltd., Tianjin, China). Weigh 1.0 g of potato skin powder into a glass tube, add 95 % 25 mL ethanol, mix thoroughly, and extract gently at 0 °C for 24 h. The filter is collected in a brown volumetric flask and adjusted to 30 mL with 95 % ethanol. Then, the absorbance of collected filter was measured at a wavelength of 665 nm and 649 nm using a UV spectrophotometer (TU-1810 Beijing Purkinje General Instrument Co., Ltd. Beijing, China). A 95 % ethanol solution is used as a blank control. Calculate the concentration of total chlorophyll according to the following formula:

$$\text{The concentration of chlorophyll A } \left(\text{mg L}^{-1}\right) C_A = 13.95\ A_{665} - 6.88\ A_{649}$$

$$\text{The concentration of chlorophyll B } \left(\text{mg L}^{-1}\right) C_A = 24.9\ A_{649} - 7.32\ A_{665}$$

$$\text{Mass fraction of the total chlorophyll } \left(\text{mg kg}^{-1}\right) = C_{(A+B)} \times 50/M$$

where: M is the weight of the sample, g.

2.7. Determination the content of SA

0.1 g of potato skin powder was added to 1 mL of pre-cooled 90 % methanol aqueous solution, and soaked overnight at 4 °C. Afterward, the mixture was centrifuged at 9180 r·min^{-1} for 10 min and collected the supernatant. The supernatant was evaporated under reduced pressure at 40 °C until no organic phase remained, followed by addition of 20 μL 1

mg mL^{-1} trichloroacetic acid aqueous solution, and mixed thoroughly for 1 min. Then, add 1 mL of ethyl acetate and cyclohexane mixture (1:1, v v^{-1}) for extraction twice. The upper organic phase was transferred to a new EP tube, dried with nitrogen gas, dissolved in 0.5 mL mobile phase, mixed, and filtered through a needle filter for analysis. Then, the SA content was detected using HPLC method mentioned by Kowalska et al. (2022).

HPLC conditions: ACCHROM S6000 high performance liquid chromatograph, Baiso C18 reversed-phase column (250 mm × 4.6 mm, 5 μm), mobile phase: methanol: 1 % acetic acid in water = 3:2, injection volume of 10 μL, flow rate of 0.8 mL / min, column temperature of 35 °C, retention time of 30 min, excitation wavelength of 294 nm, emission wavelength 426 nm, and the measurement was started after the baseline was stabilized.

2.8. Determination the browning degree of potato tubers

The browning degree was determined using spectrophotometric method with reference to the method of Dong et al. (2020). The potato tubers were peeled and cut into julienne strips, washed with deionized water to remove starch and dried with gauze, then immersed in 0.2 %, 0.4 %, 0.6 %, 0.8 % and 0.8 % $CaCl_2$ + 2 mM SA synthesis inhibitor (2-aminoindano-2-phosphonic acid, AIP) for 20 min respectively, of which deionized water was used as the control, and then taken out and wiped dry the surface water. Then these simples were immediately placed into liquid nitrogen and ground into powder using a liquid nitrogen grinder. 1 g potato powder was added to 5 mL deionized water, immediately shaken well, and left to stand at 25 °C for 10 min. Then these samples were collected at 0 h, 1 h, 2 h, 3 h, 4 h, and 5 h, centrifuged at 10,000 r·min^{-1} for 10 min at 4 °C by High-speed freezing centrifuge (TGL20MC Changsha Yingtai Instrument Co., Ltd. Changsha, China), and the supernatant was collected to measure absorbance at a wavelength of 410 nm for determining the browning degree. Each group was repeated three times.

2.9. Determination of soluble quinone content

The soluble quinone content was determined by the methods of Gao et al. (2017) and Qiao et al. (2021) with slight modifications. 1 g of potato powder was weighed and added to 5 mL methanol solution, thoroughly shaken to mix, and then ultrasonic treatment for 30 min at 27 °C. Subsequently, centrifugation was performed at 4 °C and 10,000 r·min^{-1} for 5 min. Then, 0.5 mL of the supernatant were taken and added to 1 mL of Foiln-Phenol. After the mixture reacted for 5 min at 25 °C under dark conditions, 5 mL of deionized water and 3 mL of 10 % sodium carbonate were immediately added. The mixture was then inverted to mixed thoroughly and placed in a 26 °C water bath for 1 h. The OD value measured at a wavelength of 437 nm could represent the soluble quinone content, with three repetitions each time.

2.10. Determination of PPO, CAT, and POD activity

PPO, CAT, and POD activity were determined by referring to the method of Wang et al. (2023) with slight modifications.

(1) Crude enzyme extraction: 1 g of potato powder and 0.12 g of polyvinylpyrrolidone (PVPP) were added to 5 mL of pre-cooled phosphate buffer solution (pH 6.8). The mixture was shaken evenly, and centrifuged at 10,000 r·min^{-1} for 10 min at 4 °C. The obtained supernatant was the crude enzyme solution.

(2) PPO activity determination: 0.75 mL crude enzyme solution, 1.5 mL of phosphate buffer, and 1.0 mL of 0.02 mol L^{-1} catechol solution were mixed. The absorbance values of the mixture were measured at a wavelength of 410 nm and recorded at intervals of 30 s for 3 min. A change of 0.01 in the absorbance value of the samples within each minute was taken as one unit of enzyme activity (U), and the experimental results were expressed as (U·g^{-1}·min^{-1}). The mixture solution of 2.25 mL phosphate buffer and 1.0 mL of 0.02 mol L^{-1} catechol was used as a blank control for zero calibration at a wavelength of 410 nm.

Calculation formula:

$$\text{PPO activity}\left(U\cdot g^{-1}\cdot min^{-1}\right) = (\Delta A_{410} \times V_T)/(0.01 \times F_W \times V_S \times t)$$

where: ΔA_{410} is the change of maximum absorbance value at a wavelength of 410 nm. V_T is the total volume of sample extract (mL). F_W is the mass of sample weighed (g). V_S is the volume of crude enzyme solution used in the assay (mL). t is the reaction time (min).

(3) CAT activity determination: 0.5 mL crude enzyme solution, 2.0 mL of phosphate buffer, and 0.5 mL of 0.3 % H_2O_2 were mixed. The absorbance values of the mixture were measured at a wavelength of 240 nm and recorded at intervals of 30 s for 3 min. A change of 0.01 in the absorbance value of the samples within each minute was taken as one unit of enzyme activity (U), and the experimental results were expressed as (U·g^{-1}·min^{-1}). The mixture solution of 2 mL phosphate buffer and 0.5 mL of 0.3 % H_2O_2 was used as a blank control for zero calibration at a wavelength of 240 nm.

Calculation formula:

$$\text{CAT activity}\left(U\cdot g^{-1}\cdot min^{-1}\right) = (\Delta A_{240} \times V_T)/(0.01 \times F_W \times V_S \times t)$$

where: ΔA_{240} is the change of maximum absorbance value at a wavelength of 240 nm.

(4) POD activity determination: 56 μL of guaiacol was added to 100 mL of phosphate buffer (pH 6.0), heated to dissolve, then 38 μL of 30 % H_2O_2 was added. After thorough mixing, the mixture (reaction solution) was stored at 4 °C in the dark. 0.5 mL crude enzyme solution, 0.5 mL of phosphate buffer (pH 6.0), and 2.5 mL reaction solution were mixed. The absorbance values of the mixture were measured at a wavelength of 470 nm and recorded at intervals of 30 s for 3 min. A change of 0.01 in the absorbance value of the samples within each minute was taken as one unit of enzyme activity (U), and the experimental results were expressed as (U·g^{-1}·min^{-1}). The mixture solution of 0.5 mL phosphate buffer (pH 6.0) and 2.5 mL reaction solution was used as a blank control for zero calibration at a wavelength of 470 nm.

Calculation formula:

$$\text{POD activity}\left(U\cdot g^{-1}\cdot min^{-1}\right) = (\Delta A_{470} \times V_T)/(0.01 \times F_W \times V_S \times t)$$

where: ΔA_{470} is the change of maximum absorbance value at a wavelength of 470 nm.

2.11. Determination of DPPH radical scavenging rate

DPPH solution preparation: 0.0198 g of DPPH was dissolved in a small amount of 95 % ethanol solution, and then was set volume to 250 mL.

1 g of potato powder were added to 5 mL of pre-cooled 95 % ethanol solution. The mixture was ultrasonicated at 50 °C for 30 min, and then centrifuged at 10,000 r·min^{-1} for 10 min at 4 °C. The obtained supernatant was the crude enzyme solution. 0.5 mL of crude enzyme solution was added to 2.5 mL of DPPH solution. After thorough mixing by inversion, the reaction proceeded in the dark at 25 °C for 30 min. The absorbance value measured at a wavelength of 517 nm was recorded as Ai. 0.5 mL of 95 % ethanol was inverted and mixed well with 2.5 mL of DPPH solution, then react for 30 min at 25 °C under dark condition, and

the absorbance value measured at a wavelength of 517 nm was recorded as At.

Calculation formula:

$$\text{DPPH radical scavenging rate} = (1 - \text{Ai}/\text{At}) \times 100\%$$

where: At is the absorbance value of the blank sample. Ai is the absorbance value of the sample.

2.12. Statistical analysis

All samples in this study were set with three replicates. IBM SPSS Statistics 23 software was employed for two-way ANOVA or *t*-tests. All data in the graphs are presented as mean ± standard deviation and differences between sample means were tested by Duncan's multiple comparisons (significant at $P < 0.05$).

3. Results and analysis

3.1. Transcriptomic analysis of greening and non-greening potato

In order to study the regulatory mechanism for potato greening, we performed RNA sequencing (RNA-seq) analysis of greening and non-greening potatoes. 7436 differentially expressed genes (DEGs) were found, among which 4919 DEGs were significantly up-regulated, and 2517 DEGs were significantly down-regulated (Fig. 1A). GO enrichment analysis for these DEGs revealed clusters related to calcium ion transport, cation binding, ion binding, cellular ion homeostasis, ion transport, regulation of ion transport, ion homeostasis, response to salicylic acid, salicylic acid metabolic process, cellular response to salicylic acid stimulus, and salicylic acid mediated signaling pathway (Fig. 1B). We speculated that calcium ion and salicylic acid may play a regulatory role in the process of potatoes greening.

3.2. $CaCl_2$ delays light-induced greening of potato tubers

The potatoes were soaked with different concentrations of $CaCl_2$ solution (0.2 %, 0.4 %, 0.6 %, 0.8 %, and 1.0 %), and the greening degree was observed after 3 d of light exposure. The untreated potatoes (named as CK-1) serve as the control. The results showed that the degree of potato greening treated with 0.8 % $CaCl_2$ solution was lower than that of other treatment groups (Fig. 2A). Therefore, 0.8 % $CaCl_2$ solution was selected as the optimal treatment concentration to slow down potato greening. In order to screen the optimal soaking time of $CaCl_2$, potatoes were soaked respectively in 0.8 % $CaCl_2$ solution for 5 min, 10 min, 20 min, and 30 min. The greening degree was observed after 6 d of light exposure. The results showed that the degree of potato greening treated with 0.8 % $CaCl_2$ solution for 20 min was significantly lower than that of other treatment groups (Fig. 2B, C). Additionally, the a^* values of CK-1 group were significantly ($P < 0.05$) lower than the $CaCl_2$-treated group after 3 d, 6 d, 9 d, and 12 d of light exposure (Fig. 2D). Consistently, the chlorophyll content in both CK-1 group and $CaCl_2$-treated group showed a significant increasing trend, and the chlorophyll content in $CaCl_2$-treated group was significantly lower than that in CK-1 group ($P < 0.05$) (Fig. 2E). These results indicated that $CaCl_2$ treatment could significantly inhibit the degree of potato greening and production of chlorophyll under light conditions.

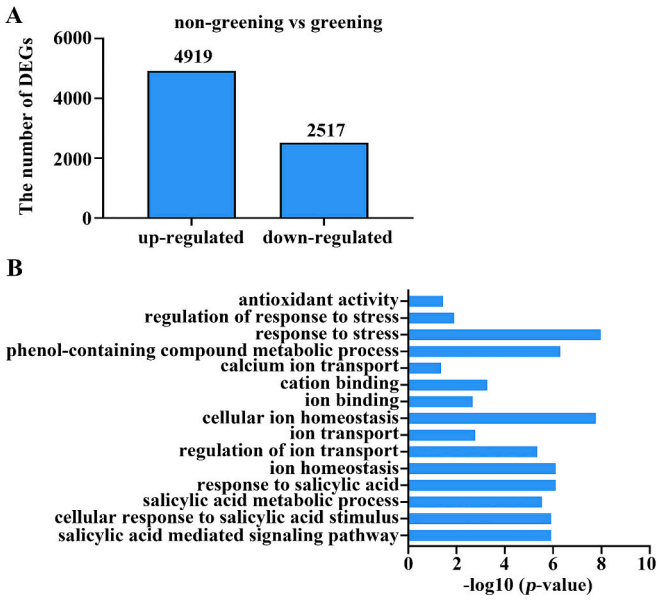

Fig. 1. RNA-seq analysis of genes differential expression between greening and non-greening potatoes. (A) Genes with differential expression between non-greening and greening potatoes. (B) GO enrichment analysis of genes with differential expression between greening and non-greening potatoes.

3.3. $CaCl_2$ affects potato greening by regulating SA synthesis

The results of RNA-seq analysis indicated that SA synthesis may also be involved in the regulation of potato greening (Fig. 1B and Table. S2). SA synthesis-related genes *ICS*, *PAL1*, *PAL2* transcript abundance increased rapidly (< 20 min) upon exogenous $CaCl_2$ treatment and respectively reached the maximum after 20 min (Fig. 3A), 20 min (Fig. 3B), and 5 min (Fig. 3C), suggesting that $CaCl_2$ may affect SA synthesis. Then the content of SA was determined in CK-1 group and $CaCl_2$-treated group. And SA content in potato epidermis can be significantly induced after exogenous $CaCl_2$ treatment (Fig. 3D). These results indicated that $CaCl_2$ regulates SA synthesis in potato epidermis.

In order to further study the mechanism of $CaCl_2$ and SA in regulating potato greening, we simultaneously applied $CaCl_2$ and SA synthesis inhibitor (AIP) and took photos at 6 d to observe the difference in the degree of potato greening compared to applying $CaCl_2$ alone. We found that after applying $CaCl_2$ and AIP, $CaCl_2$ no longer has inhibitory effects on potato greening (Fig. 3E). Moreover, the chlorophyll content in potato epidermis were higher in the simultaneously with AIP and $CaCl_2$ than in the group treated with $CaCl_2$ alone (Fig. 3F). According to the above results, we speculated that CaCl2 could delay potato greening by regulating the synthesis of SA in potato epidermis.

3.4. $CaCl_2$ affects both potato greening and enzymatic browning by regulating SA synthesis

GO enrichment analysis for 7436 DEG from the RNA-seq data of greening and non-greening potatoes also revealed clusters related to antioxidant activity, phenol-containing compound metabolic process, response to stress and regulation of response to stress (Fig. 1B). And 3 polyphenol oxidase (PPO) genes (Table. S3) and 16 peroxidase genes were screened from 7436 DEG (Table. S4). The enzymatic browning reaction is the catalysis of phenolic substances by PPO into colored quinone substances (Moon et al., 2020). Peroxide content in fresh-cut products is also one of the important factors affecting the degree of enzymatic browning (Ma et al., 2021). So, we mashed the non-greening and greening potato tubers into slurry and left them at 24 °C for different times (0 h, 1 h, 2 h, 3 h, 4 h and 5 h), then detected the degree of browning. The results showed that the browning degree of greening potato slurry is consistently higher than in non-greening potato slurry (Fig. 4A, B), indicating a positive correlation between greening and enzymatic browning. Potato greening is the conversion of amyloplasts into chloroplasts under light conditions (Tanios et al., 2018). Enzymatic browning refers to the production of colored quinone compounds from phenolic substrates with the participation of PPO and oxygen after cellar damage occurs (Serra et al., 2021). And the key enzyme PPO is activated into active PPO in the chloroplast (Shi et al., 2024). It can be seen that

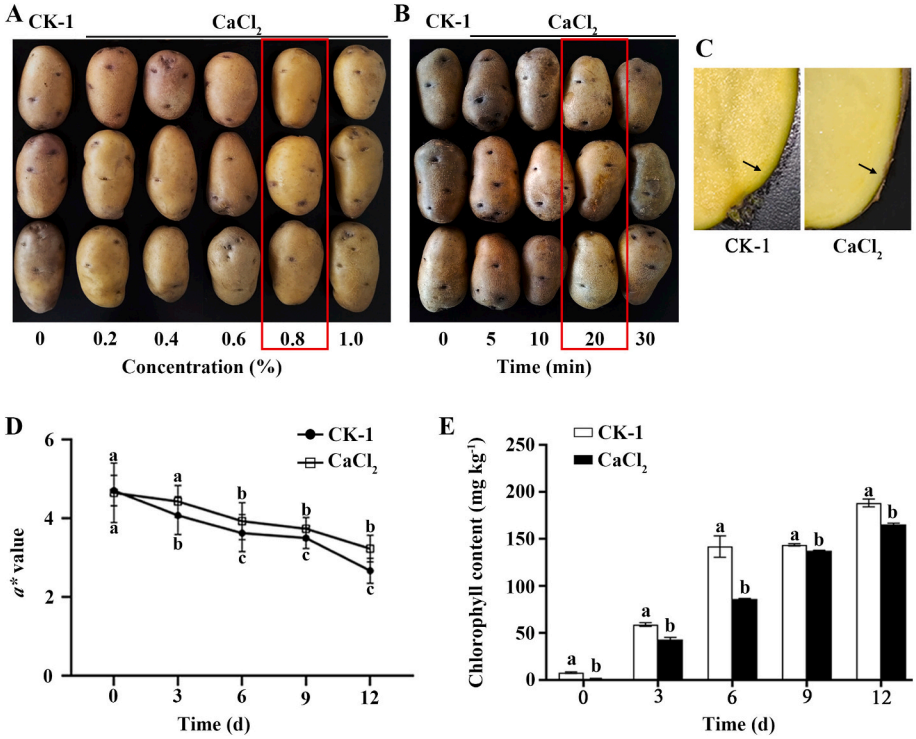

Fig. 2. $CaCl_2$ treatment delays greening of potato tubers. Screening for optimal $CaCl_2$ treatment concentrations (A) and time (B). (C) Potatoes phenotype observation of untreated group (CK-1) and $CaCl_2$-treated group (0.8 % $CaCl_2$, 20 min) after exposure to light (A was photographed at 3 d of light, and B and C were photographed at 6 d of light). (D) is the *a* *value in CK-1 and $CaCl_2$-treatd groups after 0 d, 3 d, 6 d, 9 d, and 12 d of light exposure. (E) The chlorophyll content of potato epidermis in CK-1 and $CaCl_2$-treated groups after 0 d, 3 d, 6 d, 9 d, and 12 d of light exposure. Different letters indicate statistically significant differences ($p < 0.05$) and error bars represent SD (Saliency Detection) ($n = 3$).

chloroplasts are the important organelles for both browning and greening. We speculated that after the amyloplasts of potato tubers transform into chloroplasts, it is more conducive to the occurrence of enzymatic browning reactions.

Studies have showed that treating fresh-cut *Luffa cylindrica* with 1 % $CaCl_2$ can inhibit enzymatic browning (Feng, Feng, et al., 2022; Feng, Sun, et al., 2022). Consistently, we also found that the enzymatic browning of potatoes after treatment with different concentration (0.2 %, 0.4 %, 0.6 %, and 0.8 %) of $CaCl_2$ was significantly inhibited, with the best inhibitory effect observed with 0.8 % $CaCl_2$ treatment (Fig. S1). $CaCl_2$ can simultaneously inhibit both potato greening and enzymatic browning. To investigate whether $CaCl_2$ also regulates potato enzymatic browning by affecting SA content, we simultaneously applied both $CaCl_2$ and SA synthesis inhibitor (AIP) to observe differences in potato browning degree compared to applying $CaCl_2$ alone. We found that after applying $CaCl_2$ and AIP, the inhibitory effect of $CaCl_2$ on potato browning was weakened (Fig. 4C). These results suggested that $CaCl_2$ can regulate both potato greening and enzymatic browning by affecting SA synthesis.

3.5. SA inhibits enzymatic browning by enhancing antioxidant capacity and suppressing PPO activity in potatoes

To detect the role of SA in enzymatic browning, we treated fresh-cut potato strips with 100 μM SA, then ground them into slurry to measure the degree of browning. The results showed that with increasing time of storage (0 h, 1 h, 2 h, 3 h, 4 h, and 5 h), the browning degree of potato slurry in the control (CK-2) group remained higher than that of the SA-treated (SA) group (Fig. 5A). Consistently, the content of colored quinone, the product of enzymatic browning reaction, was higher in CK-2 than in the SA group (Fig. 5B). Further research has also found that SA treatment can significantly inhibit the activity of the key enzyme PPO in the enzymatic browning (Fig. 5C). We speculated that SA treatment can reduce quinone production by inhibiting PPO activity, thereby suppressing the degree of enzymatic browning.

Moreover, the activities of antioxidant enzymes (CAT and POD) in the CK-2 group remained lower than that of the SA group (Fig. 5D, E). And the DPPH inhibition rate of CK-2 group was also lower than that of the SA group (Fig. 5F). Greater antioxidant capacity leads to lower levels of membrane lipid peroxidation, making the cell membrane structure more stable and substrates, oxygen, and browning enzymes such as PPO less likely to come into contact with each other, thus slowing down the level of browning. Therefore, we speculated that SA treatment can also enhance the total antioxidant capacity of potato tubers by increasing the activity of CAT and POD, thereby inhibiting enzymatic browning.

4. Conclusion

In the study, we found that treating postharvest potatoes with 0.8 % $CaCl_2$ for 20 min significantly delays potato greening under light exposure. Furthermore, $CaCl_2$ promotes the synthesis of SA in potatoes. When potatoes were co-treated with $CaCl_2$ and SA synthesis inhibitor AIP, the delaying effect of $CaCl_2$ on potato greening disappeared, indicating that $CaCl_2$ alleviated potato greening by influencing endogenous SA synthesis. We also observed that greening potatoes exhibited more severe enzymatic browning compared to non-greening potato tubers, suggesting a positive correlation between greening and browning. Interestingly, $CaCl_2$ can also inhibit enzymatic browning by promoting internal SA synthesis. These results indicated $CaCl_2$ and SA can regulate both potato greening and enzymatic browning, serving as a crucial link between these two biological processes in potato tubers (Fig. 6).

CRediT authorship contribution statement

Jingkui Shi: Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Wenxin Xie:**

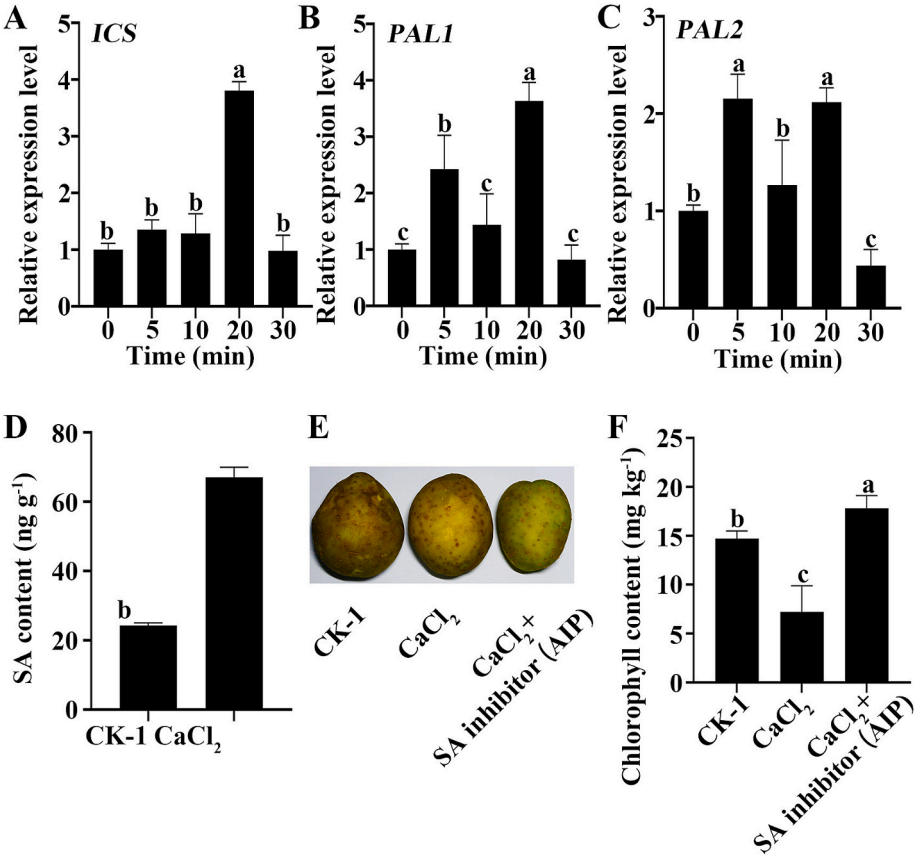

Fig. 3. $CaCl_2$ inhibits potato greening by increasing the content of SA in potatoes. (A, B, C, and D) The treatment of $CaCl_2$ affects the expression level of SA synthesis-related genes *ICS* (A), *PAL1* (B), and *PAL2* (C), and the content of SA (D) on potato epidermis. (E) Phenotype observation of potato greening in the untreated group (CK-1), $CaCl_2$-treated group ($CaCl_2$), and the group ($CaCl_2$ + SA inhibitor AIP) treated with a combination of $CaCl_2$ and SA synthesis inhibitor (AIP). (F) after 6 d of light exposure, the chlorophyll content, in potato epidermis of CK-1, $CaCl_2$, and $CaCl_2$ + SA inhibitor AIP group were measured. Different letters indicate statistically significant differences ($p < 0.05$) and error bars represent SD (Saliency Detection) ($n = 3$).

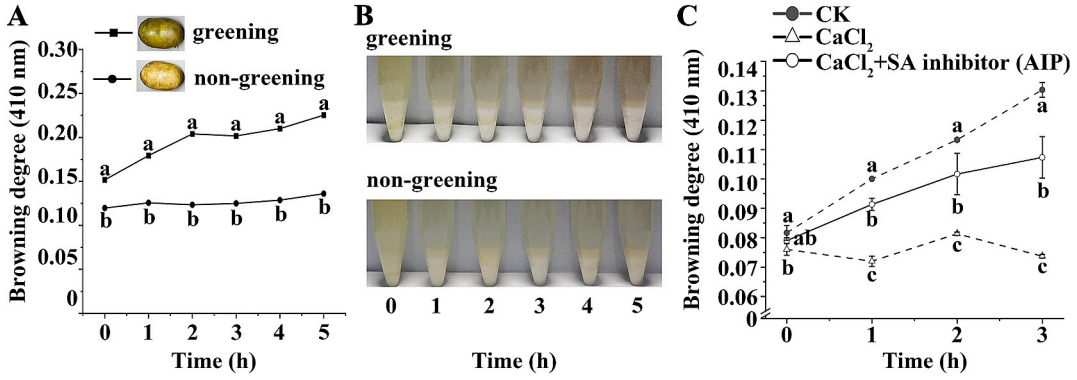

Fig. 4. (A) Browning degree of greening and non-greening potato slurry at 24 °C for different times (0 h, 1 h, 2 h, 3 h, 4 h and 5 h). (B) is the phenotype observation of (A). (C) At different time points (0 h, 1 h, 2 h, and 3 h) under 24 °C, the degree of potato slurry browning was compared between CK, $CaCl_2$ group (0.8 % $CaCl_2$), and the $CaCl_2$ + SA inhibitor (AIP) group. Different letters indicate statistically significant differences ($p < 0.05$) and error bars represent SD (Saliency Detection) ($n = 3$).

Writing – original draft, Validation, Supervision, Investigation, Data curation. **Yanmei Sun:** Writing – original draft, Conceptualization. **Qingyu Shi:** Writing – review & editing. **Xin Xing:** Writing – review & editing. **Qingguo Wang:** Writing – review & editing. **Qingqing Li:** Writing – review & editing, Project administration, Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

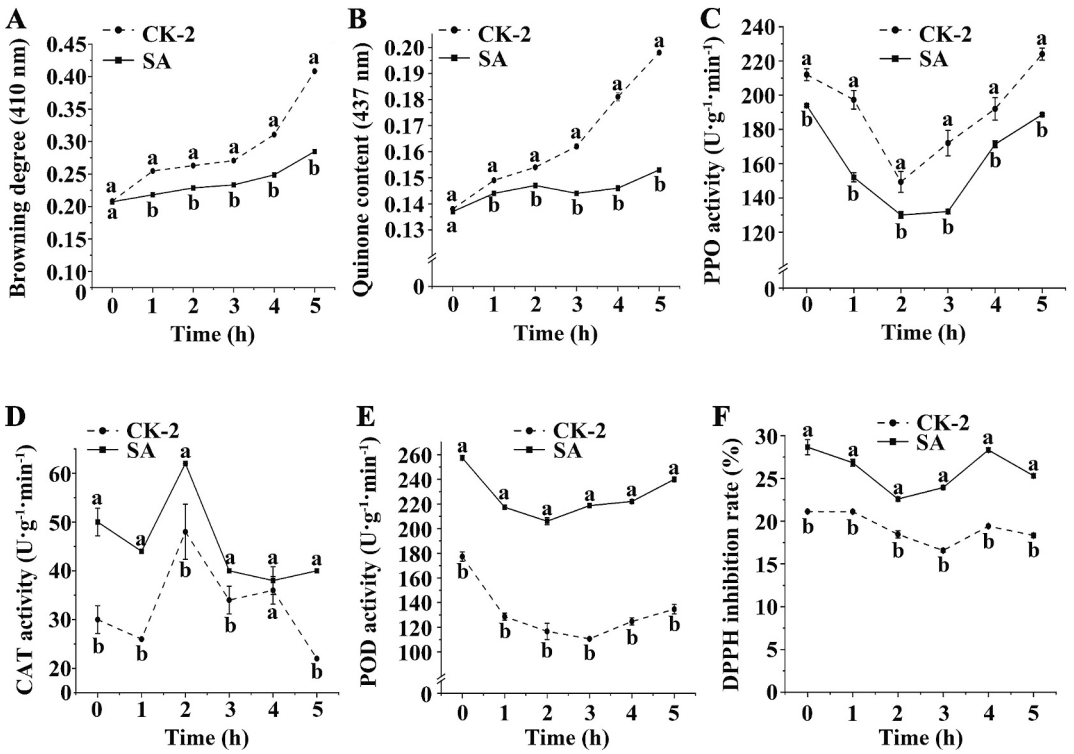

Fig. 5. The browning degree (A), quinone content (B), PPO activity (C), CAT activity (D), POD activity (E), and DPPH inhibition rate (F) of potato slurry in the control (CK-2) and SA-treated (SA) group at different time points (0 h, 1 h, 2 h, 3 h, 4 h, and 5 h) under 24 °C. Different letters indicate statistically significant differences ($p < 0.05$) and error bars represent SD (Saliency Detection) ($n = 3$).

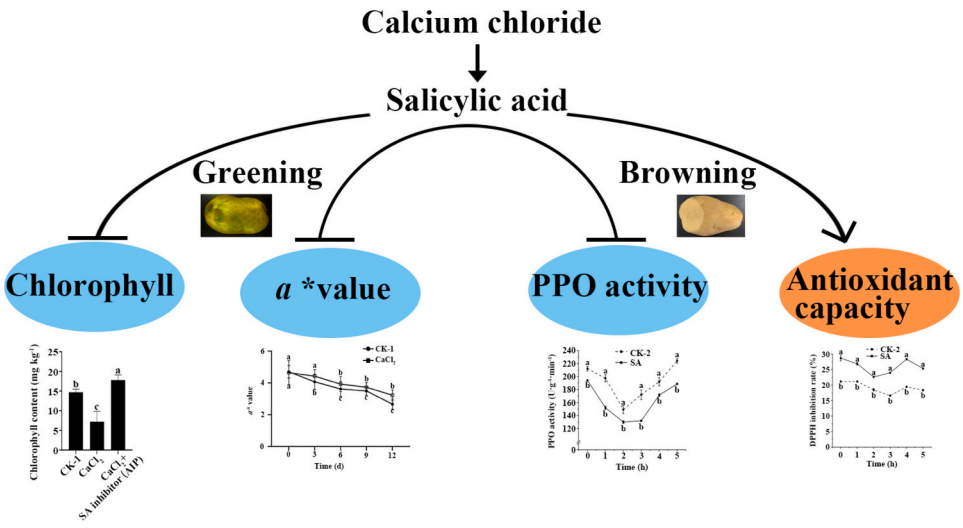

Fig. 6. Mechanism of cacl2 regulation of browning and greening in potato.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by grants from the Potato Industry Innovation Team for Modern Agricultural Industry Technology System, Shandong Province (SDAIT-16-11), the National Natural Science Fund of China (31800230), and Natural Science Foundation of Shandong Province (ZR2023MC051). The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochms.2024.100229.

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