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Effect of Calcium Chloride Treatment on Quality of Cowpea (*Vigna unguiculata* (L.) Walp)

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Summary

The chill-sensitive cowpea cv 'Guinong' had subjected to different pre- and postharvest calcium treatments in order to determine an effective means of increasing shelf life, reducing the incidence and severity of rust stains. However, treatment of postharvest calcium chloride dipping had found to be less effective in reducing the rust stains symptoms. 'Guinong' were foliar sprayed with 0.2 % calcium chloride solution, and harvested at the 30–50 % stage of maturity. At harvest, treated pods had lower soluble protein and malondialdehyde (MDA) than control, while no effect on chlorophyll content and the time required ripening. During low temperature storage, lower levels of decay rate, weight loss, soluble protein, chlorophyll content, peroxidase (POD) activity and MDA has found in treated pods compared with control. In addition, the storability of treated pods had extended for up to 15 days more than that of the control and the preharvest treatment prior to postharvest.

Key words. cowpea (cv. 'Guinong') – treatment – calcium chloride – quality

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is a drought-tolerant crop widely cultivated throughout the tropics and subtropics, covering Asia, Africa, central and south America, as well as southern Europe and the USA (Smart 1976). The estimated average worldwide production of Cowpea is 5.39 million metric tons annually (Fao 2010). It has similar physical and chemical properties as common beans (*Phaseolus vulgaris*), except for its fat content, which takes up 9 to 10 % of Cowpea (Samman et al. 1999). Cowpea seeds are rich in protein (18–35 %),and also contain valuable amounts of fiber as well as vitamins and minerals. Since it has a good nutritional quality (Onwuliri and Obu 2002), it is widely used in human foods.

However, the storability of Cowpea is quite poor due to tender pods organization and high respiration rate. If not treated after harvest, within short time it will wilt, fade and rot. Therefore, the supply of cowpea is highly seasonal and regional. Postharvest grain storage, however, is a major constraint for crop expansion and year-round availability. The 25 % loss rates had used in the baseline analysis and the impact of the higher rate of 50 % had evaluated through sensitivity testing (Moussa et al. 2011). At cold-storage treatment (10 °C), the total moisture of *Vigna sinensis* fruit have well maintained, the content of vitamin C, chlorophyll, rates of respiration decreased, and increased in cell membrane. (Chen 2006; Zhang et al. 2011).

Calcium is an important factor in the cell membrane structure as well as in the cell wall structure. Stabilizes pectin-protein complexes of the middle lamella of the cell wall have served as an intermolecular binding agent. In particular, calcium treatment, either by preharvest foliar sprays or by postharvest dips, represents a safe and potentially effective method for increasing the storage life and preserving the quality of some fruits. It has proposed that Ca^{2+} plays an important role in delaying fruit senescence through stabilization of cell membranes and delaying membrane lipid catabolic processes during postharvest storage (Serrano et al. 2004). Preharvest spraying with calcium improved the production of H_2O_2 as well as bioactive components such as anthocyanin, phenolics and flavonoid of raspberry fruit, while total antioxidant ability, DPPH radical and hydroxyl radical scavenging ability were significantly inhibited during postharvest storage (Li et al. 2009).

The present study had carried out to determine the effect of different calcium treatment on quality of cowpea and to investigate the relationship between calcium treatment and storability during 8 ± 1 °C storage.

Materials and Methods

Plant material

Field experiments had done on South Campus Farm Huaxi of Guizhou University located in Guiyang, China. One hundred plants have selected for cowpea cv 'Guinong'. One-third plants treated with 0.2 % Calcium chloride at 6:00 on September, 22nd, 2011 (4 days before harvest). The remaining plants have sprayed with distilled water. All plants grown on the one conditions, including irrigation, fertilization and phytosanitary treatments. 'Guinong' pods harvest at the commercial ripening stage according to the external appearance of the pods. Then 'Guinong' pods (from both treated and untreated plants) transferred to the laboratory. Half the fruits by spraying with distilled water were dipped in 0.2 % Calcium chloride solution for 30 min and spread on table, the remaining fruits were used as controls (CK), and where 100 'Guinong' pods of each treatment were selected based on their colour, size uniformity and absence of mechanical damage. Afterwards the 'Guinong' pods were packed separately in plastic bags and stored at 8 ± 1 °C in a temperature-controlled chamber in permanent darkness and with a relative humidity of 85 %–90 %. 1, 5, 11, 15 and 19 d after harvesting, 10 'Guinong' pods per treatment were randomly removed from storage and used for analyses. Each treatment was performed in triplicate.

Decay rate and weight loss measurements

The decay rate of 'Guinong' pods assessed using a scale developed by Li et al. (2011). The decay rate was scored on a 0–3 scale where 0 = none, 1 = slight (0–25 % surface affected), 2 = moderate (25–50 % surface affected), and 3 = extreme (> 50 % surface affected). The decay rate had calculated as 100 ×∑ (decay score × pods within each class)/(total pods × the highest score).

For determining weight loss, at each sampling, 20 pods per treatment were weighed individually (to 0.01 g) immediately after harvest and after the different storage periods, per treatment in triplicate. Results were recorded as the mean ± SE and expressed as the percentage weight loss with respect to the initial weight, respectively.

Soluble protein measurements

Soluble protein content of pod measured according to coomassie brilliant blue G250 method (Read and Northcote 1981). The pod about 5.0 g (50 % seeds plus 50 % hulls) were ground into homogenate in the mortar with 25 ml distilled water, then The homogenate was centrifuged at 12,000×g for 20 min at 4 °C by Sorvall Ledend Micro 17R centrifuge (model: 17R, Thermo Fisher Scientific Inc, Shanghai, China). The supernatant was used for assay of soluble protein content using Colorimetric Determination with T6-spectrophotometer (model: T6, Beijing purkinje general instrument, Beijing, China) at 595 nm colorimetric wavelength.

Chlorophyll measurements

Chlorophyll was determined using a colorimetric method (Cao et al. 2007). The pod about 5.0 g (50 % seeds plus 50 % hulls) were ground into homogenate in a mortar with a small amount of quartz sand, calcium carbonate powder and 5 mL 80 % acetone, then add 10 mL 80 % acetone, until the organization became to white, standing about 3–5 min. The homogenate filtered into 50 mL brown volumetric flask through filter paper. The mortar, pestle and the residue were rinsed several times with 80 % acetone, until the filter paper and residue no green. The filtrate diluted in 80 % acetone until a volume of 25 ml. The supernatant was used for assay of chlorophyll using Colorimetric Determination with T6-spectrophotometer (model: T6, Beijing purkinje general instrument, Beijing, China) at 663and 645 nm colorimetric wavelengths, repeated three times.

POD activity measurements

POD activity in pod estimated by using the method (Cao et al. 2007). Pod were weighed (5.0 g, 50 % seeds plus 50 % hulls) and grounded with a pestle in an ice-cold mortar with 3 ml extracting solution (1 mmol PEG, 4 % PVPP and 1 % Triton X-100).The homogenates were centrifuged at 12,000×g for 30 min at 4 °C.The resulting supernatants were used for assay of enzymatic activities. Crude enzyme extraction solution was assayed using 6.0 ml 25 mmol L^{-1} guaiacol as the substrate. Add 500 μl enzyme extraction and 400 μl 0.5 mol L^{-1} H_2O_2, the reaction started by rapid mixing. At start time, the reaction mixture into the cuvette, placed in the spectrophotometer sample chamber. With distilled water as a reference, the absorbance values were record at 470 nm when the reaction up to 15 s, as the initial value, and these monitored by measuring the change of absorbance at 470 nm for 1 min. The speci c activity was expressed as U mg ^{1} fresh pods, where 1 unit was defined as increase 0.001 ΔOD_{470}/ min g F_W.

Measurement of malondialdehyde content

Malondialdehyde (MDA) content were determined by the thiobarbituric acid (TBA) reaction updated from the method as described by Dipierro and Leonardis (1997). In brief, 5.0 g tissue (50 % seeds plus 50 % hulls) homogenized in 10 mL 100 g L^{-1} trichloroacetic acid. The homogenate centrifuged at 12,000 × g for 20 min at 4 °C. To a 3 mL the supernatant, 3 mL 6.7 g L^{-1} thiobarbituric acid added. The mixture boiled at boiling water bath for 20 min and cooled immediately, and the absorption of the supernatant read at 450, 532 and 600 nm, respectively.

Statistics

Statistical tests were performed using the SPSS® computer program, version 16.0 (SPSS Statistical Software, Inc., Chicago, IL, USA) to determine differences at the 5 % level between treatments over storage time using a one-way analysis of variance (ANOVA). If a significant difference was observed, Duncan's test was performed to evaluate the significance of differences among mean values. In the case of sensory evaluation, a nonparametric ANOVA of Kruskal-Wallis Test and Mann-Whitney U-test was used. Results were presented as mean ± standard error.

Results

Decay rate and weight loss

No visible rot observed in Mauritius cowpea during the first 5 days of storage, then the rust stains began to appear and obverse at the 11th day of storage. Similar variations had also found by Chen (2006), which irrespective of the treatments applied. Fig. 1 shows the decay rate of 'Guinong' pod with calcium chloride treatment and storage time. During storage, decay rate of 'Guinong' pod increased slowly at first. However, during the 11th to 19th day of storage, the increment of decay rate became much faster, and less slow by the calcium chloride treatment, and preharvest less than postharvest.

Weight loss increased during storage, the increase being over the 11 days of shelf life at 8 ± 1 °C, although treated pods exhibited lower weight loss than control (Fig. 1). As shown in Fig. 1, the postharvest calcium chloride treatment did not affect the pods weight loss, with no differences among different treatments. The effect of calcium chloride treatment on the weight loss of 'Guinong' pod during storage reveals that calcium chloride treatment reduced pods weight loss, and this protective effect was especially remarkable for preharvest calcium chloride treatment. Thus, after 15 days of storage, weight loss in control pods was 32.2 %, while in treated pods were 22.4 and 28.9 % for preharvest and postharvest respectively.

Soluble protein and chlorophyll content

For the treatment and control, the soluble protein contents both showed the trend of first rise and then fall during storage at 8 °C. At every sampling date, the value in control was higher than that of treatment, and the effect of preharvest calcium chloride treatment optimum. As shown in Fig. 2, the calcium chloride treatment can delay the transformation of other substances to soluble protein during storage. The control was significantly greater ($P < 0.005$).

The physical parameters evaluated showed signi cant differences under the calcium chloride treatments studied for 'Guinong' pod (Fig. 2). The difference in the seed coat colours of the cowpea confirmed by the variation in the total chlorophyll during storage. The chlorophyll content had gradually reduced during the storage, and was significant at the 5th day of storage, then relatively slowed. The preharvest treatment has been higher than the postharvest treatment and the control was signi cantly ($P < 0.05$) lower than treatment. Thus indicated that the preharvest calcium chloride treatment has a significantly role to maintain chlorophyll content.

POD activity and MDA content

POD activity gradually increased in both treated and control pods stored at 8 ± 1 °C, but then POD activity in calcium chloride treated pods decreased from the 11th days of storage, with a significant difference between the preharvest and postharvest (Fig. 3). POD activity in control pods increased to 3132.468 ΔOD_{470}/min g at 11 days and then gradually decreased, which illustrated that the peroxide of 'Guinong' pod gradually increased during

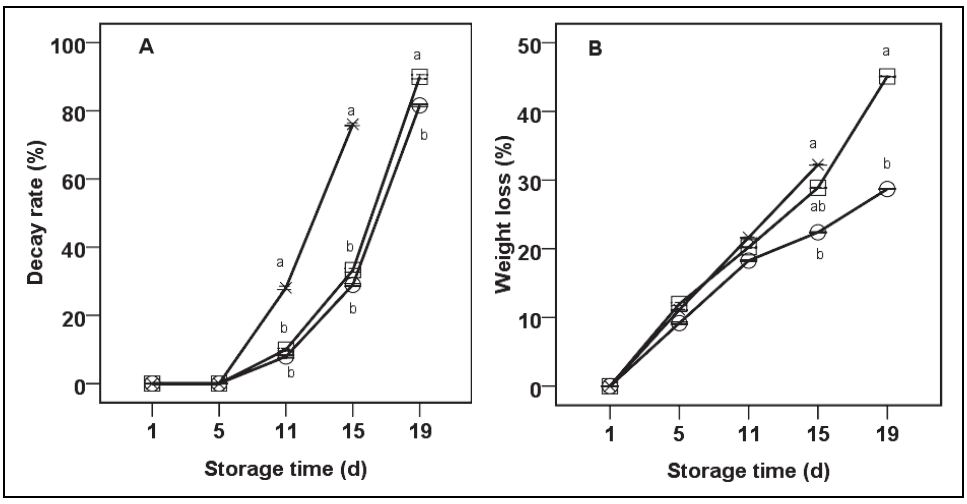


Fig. 1. Effect of pre and postharvest calcium chloride treatment on decay rate (A) and weight loss (B) of 'Guinong' pods during storage at 8 °C. Date are expressed as means of triplicate assay. ○ Preharvest treatment, □ Postharvest treatment, × Control.

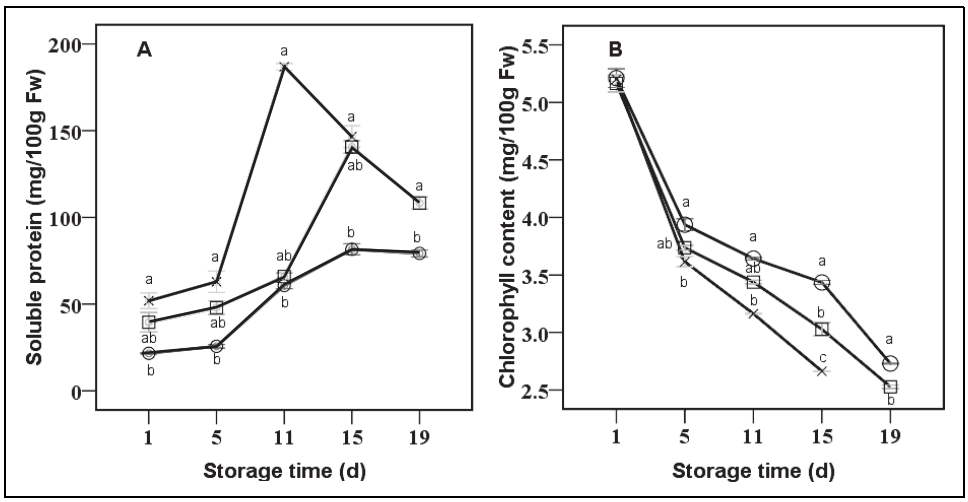


Fig. 2. Effect of pre and postharvest calcium chloride treatment on soluble protein (A) and chlorophyll content (B) of 'Guinong' pods during storage at 8 °C. Date is expressed as means of triplicate assay. ○ Preharvest treatment, □ Postharvest treatment, × Control; Vertical bars mean ± SE of three replications

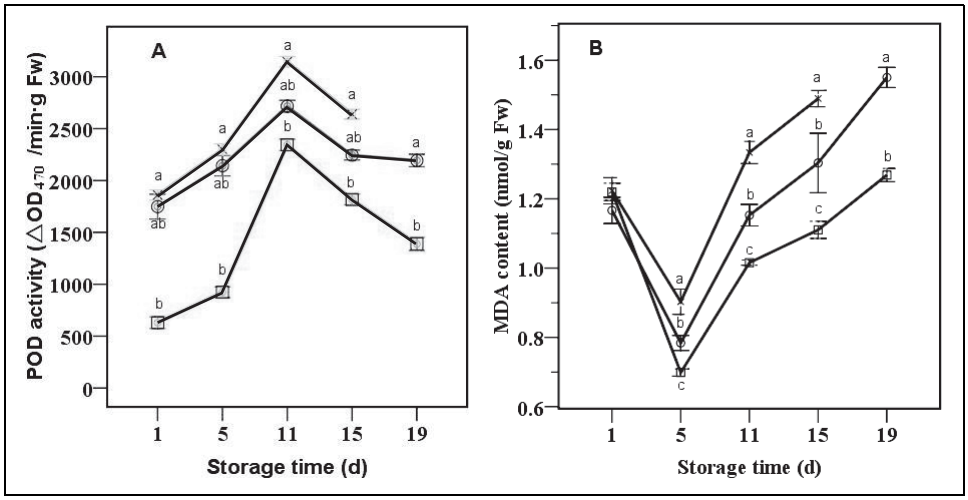


Fig. 3. Effect of pre and postharvest calcium chloride treatment on POD activity (A) and MDA content (B) of 'Guinong' pods during storage at 8 °C. Date are expressed as means of triplicate assays. ○ Preharvest treatment, □ Postharvest treatment, × Control, Vertical bars mean ± SE of three replications.

storage and stimulated the increment of The POD activity, and decreased. POD activities of 'Guinong' pod preharvest treatment were significantly ($P < 0.001$) lower than those of control and postharvest and its curve is more gentle, and both showed significant difference on treatment ($P < 0.001$). Thus means that the oxidation disorders of 'Guinong' pod were suppressed by preharvest calcium chloride treatment.

Lipid peroxidation and membrane injury have indicated by measuring the content of MDA, because MDA is a secondary product of polyunsaturated fatty acid oxidation. As shown in Fig. 3. For the 'Guinong' pod under the calcium chloride treatment and control, the MDA contents decreased sharply and almost half within 5 days of storage and then gradually increased. In contrast, the MDA contents of the control decreased slowly and increased quickly during storage. No significant differences had statistically found in the contents of MDA between pre- and postharvest treatment. However, MDA content of postharvest treatment significantly ($P < 0.05$) lower than the control. The degradation of the polyunsaturated fatty acids due to peroxidation produces MDA at the same time inducing membrane rigidification and death of cells. Therefore, membrane lipid peroxidation may be one of the first events in the manifestation of chilling injury.

Discussion

The protection of calcium against fungal contamination by reinforcing pods tissues, the increment of decay rate and less slow by the calcium chloride treatment. (Lara et al. 2004; Chen et al. 2011). Calcium chloride treatment could strongly suppress the decay rate of apple, strawberry and litchi (Moor et al. 2006; Chaiprasart et al. 2006; Zhou et al. 2009; Hussain et al. 2012; Irfan et al. 2013). The reduction in pods weight loss had observed during cold storage of peaches and nectarines pods from plants sprayed with calcium chloride (Serrano et al. 2004). Positive effects of postharvest calcium chloride treatment have obtained in tomato and apple in terms of maintenance of water rate (Omaima and Karima 2007; Chen et al. 2012).

Calcium chloride treatment significantly increased the cell wall material and uronic acid, significantly reduced protein content and water soluble of cell wall polysaccharide of pear and strawberry (YAN et al. 2010; ZHANG et al. 2011). The preharvest calcium chloride treatment has a significantly role to maintain chlorophyll content in the preservation of Chinese peony cut flowers (TANG et al. 2009). This observation may be due, at least partially, to the changes in the polyphenolic compounds, ascorbic acid, and carotene present in the whole seeds and in the starch fractions, as reported (GALVEZ and RESURRECCION 1993; SHIMELIS et al. 2006). However, in other fruits such as strawberry and papaya, treatment with Ca^{2+} during fruit growth delayed the occurrence of the typical red and orange colour of these fruits (LARA et al. 2004; AL-ERYANI-RAQEEB et al. 2009), effectively restrain yellowing of Pak-choi (SHENG et al. 2007).

Calcium chloride could induce of the POD activities in Loquat Fruit (ZHENG 2009) and sweet cherry (MOHINI 2007) before harvest. Calcium treatment before harvest had positive effect on the anti-senescence ability of postharvest Dongzao fruit (SHI et al. 2004). Changes in MDA levels in a tissue can be a good indicator of the structural integrity of the membranes of plants subjected to low temperature (POSMYK et al. 2005). Preharvest calcium treatment can reduced MDA contents, the peel pitting of moonlight plum fruit and 'Fengjie' Navel Orange, and positively increased activities of membrane lipid peroxidation protective enzymes during storage, reducing the pitting incidence (YUAN et al. 2010; HAN et al. 2010).

The preharvest spraying with 0.2 % calcium chloride solution was effective in increasing fruit growth and improving pods nutrition before harvest without any toxic or detrimental effects to 'Guinong' pods or delay in the ripening process. At harvest, treated pods had lower soluble protein and MDA than control. During low temperature storage, lower levels of decay rate, weight loss, soluble protein, chlorophyll content loss, POD activity and MDA had found in treated 'Guinong' pod compared with control. The foliar sprays had similar effects to the postharvest application of calcium chloride in maintaining pods quality during storage. Since postharvest treatments require speci c machinery and are often very expensive, this preharvest application could have several advantages for large-scale operations.

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