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Effect of Various Calcium Salts for the Prevention of the Onion (*Allium cepa* L.) Tissue Softening

Ok-Ju Kang†

Professor, Dept. of Food and Nutrition, Kyungnam University, Changwon 51767, Republic of Korea

ABSTRACT

This study examined the effects of different calcium salts—calcium carbonate, calcium hydroxide, and calcium chloride—on the softening of onion tissues during storage. Specifically, it focused on analyzing the enzymatic polygalacturonase (PG) and pectin methylesterase (PE) activities, calcium ion concentrations, tissue strength, hardness, and color changes in onions treated with various concentrations of these calcium salts. Calcium carbonate at a concentration of 70 mM was the most effective at inhibiting PG activity, reducing it by approximately 4.4 times compared to untreated onions after 60 days of storage. Calcium hydroxide also reduced the PG activity significantly, with the 90 mM treatment being the most effective, albeit less impactful than calcium carbonate. Calcium chloride at 20 mM lowered the PG activity by 2.8 times but was less effective than calcium carbonate. PE activity varied according to the type and concentration of the calcium salt used. Calcium chloride at 20 mM was the most effective in enhancing the PE activity. In terms of the calcium ion concentration, calcium chloride showed the highest levels of penetration, with the 60 mM treatment group reaching a peak concentration of 33.435 mM after 30 days of storage. The strength and hardness tests showed that calcium chloride consistently maintained the highest strength and hardness levels over the storage period, particularly at 40 mM and 90 mM. Regarding the color changes, the L*, a*, and b* values indicated that onions treated with calcium carbonate closely resembled the control group in terms of color stability, while those treated with calcium chloride retained better overall color throughout storage. Therefore, among calcium carbonate, calcium hydroxide, and calcium chloride, treatment with 20 mM calcium chloride resulted in the most effective inhibition of onion tissue softening.

Key words: onion, softening, calcium salts, polygalacturonase, pectin methylesterase

INTRODUCTION

Onion (*Allium cepa* L.) is an ancient and valuable vegetable/field crop needed for people's nutrition and medicine worldwide. As a vegetable crop, it ranks second after tomato production (Shannon MC & Grieve CM 1999; Ashraf M & Harris PJC 2004). Onion is mainly used for flavoring food formulations but as well as for its high nutritional and therapeutic potentialities due to its important nutrients and health-promoting phytochemicals contents (Goudra GP *et al* 2018). Beside, onion also serves as potential medicinal purposes for cardiovascular disease, cancer, cataract, thrombolis, blood pressure and headache, anemia, urinary disorders, bleeding piles, and teeth disorders (Albishi T *et al* 2013). Moreover, onion is suspected to show antioxidant, antidiabetic, antitumor, antihypercholesterolemia, antiulcer, platelet anti-aggregating, natural antibrowning properties, and to be a good appetizer and food digester (González-Peña D *et al* 2015; Goudra GP *et al* 2018; Hussein JB *et al* 2018). These numerous properties are presented onion as a good candidate for designing functional foods. However, since onion has high moisture content, its storage stability is very weak and the softening phenomenon of tissue breaks down, resulting in heavy weight loss and corruption during storage period (Khan MKI *et al* 2016; Goudra GP *et al* 2018).

Softening is a major determining factor of plant storage quality (Wang D *et al* 2018). Ca, as a nontoxic and environmentally friendly treatment agent, has a profound effect on fruit ripening by altering pectin deesterification and Ca cross-link formation in physical properties of the cell wall, including strength and elasticity, cell wall loosening, and swelling (Hocking B *et al* 2016; Gao Q *et al* 2019). The plant cell wall is composed of a primary wall, intercellular layer, and secondary wall. The intercellular and middle layers are mainly composed of pectin polymers (Aghdam MS *et al* 2012), and Ca partakes in the constitution of the intercellular layer to form pectin-Ca. Ca treatment delayed fruit ripening in 'Chilean'

† **Corresponding author :** Ok-Ju Kang, Tel: +82-55-249-2235, Fax: +82-505-999-2104, E-mail: koj117@kyungnam.ac.kr

strawberries by preventing the normal degradation of the cell wall during cold storage (Figueroa CR *et al* 2012). Preharvest $CaCl_2$ treatment effectively delayed apple softening by inhibiting cell-wall degrading enzymes and enhancing pectin crosslinks (Gao Q *et al* 2019). The activities of pectin methylesterase (PE), polygalacturonase (PG), pectin lyase (PL), and β-galactosidase (β-Gal) are positively correlated with the soluble fraction of the cell wall, but negatively correlated with fruit firmness (Pitzschke A *et al* 2016). Additionally, Ca application can effectively inhibit decreased fruit firmness, reduce fruit respiration rates, ethylene production, and cell wall degrading enzyme activities, maintain cell wall structure stability, reduce the occurrence of physiological diseases, prolong the storage period, and promote fruit quality (Mahajan BVC & Dhatt A 2004; Lötze E *et al* 2008; Zhang Z *et al* 2022). Moreover, different calcium salts have been studied for decay prevention, sanitation and nutritional enrichment of fresh fruits and vegetables. Calcium carbonate and calcium citrate are the main calcium salts added to foods in order to enhance the nutritional value (Brant LA 2003). Other forms of calcium used in the food industry are calcium lactate, calcium chloride, calcium phosphate, calcium propionate and calcium gluconate, which are used more when the objective is the preservation and/or the enhancement of the product firmness (Luna-Guzmán I & Barrett DM 2000; Alzamora SM *et al* 2005).

Therefore, the objective of this study is to investigate the effect of different calcium salts (calcium carbonate, calcium hydroxide, and calcium chloride) on the softening of onion tissue. The enzymatic activities of PG and PE, Ca^{2+} concentration, strength, hardness, color changes of onion tissue for storage periods after different calcium salts with different concentration were investigated.

MATERIALS AND METHODS

1. Chemicals and Reagents

Calcium carbonate, calcium hydroxide, and calcium chloride were purchased from ES Food Materials (Gyeonggi-do, Korea). Sodium chloride, polygalacturonic acid, citric acid, cyanoacetamide, ammonium sulfate, pectin, permanganate, sodium arsenite, mono-*d*-galacturonic acid, and pentan-2,4-dion were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All other chemicals utilized in this study were of analytical grade.

2. Sample Preparation

After removing the outer skins, the onions were washed with distilled water. Subsequently, they were cut into pieces measuring 3 cm × 3 cm, and preference was given to pieces with a thickness exceeding 5 mm. Following this, 20 g of the selected onions were placed into glass bottles with screw caps, each having a total volume of 120 mL. These bottles were then filled with a calcium solution and stored at a constant temperature of 25℃ for 15, 30, 45, and 60 days in a constant temperature chamber. The concentrations of calcium salts were as follows: calcium carbonate (30, 50, 70, 90 mM), calcium hydroxide (30, 60, 90, 120 mM), and calcium chloride (10, 20, 40, 60 mM). While calcium hydroxide and calcium chloride were prepared with distilled water at each concentration, calcium carbonate, insoluble in distilled water, was prepared using 5% citric acid. Additionally, a 0.2M NaCl group was employed as a control for calcium salt treatment. As per the safety evaluation by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the acceptable daily intake (ADI) for calcium salts is designated as 'Not Specified (NS)'. Although specific usage standards are lacking in Korea, it is generally recommended to add calcium to food at less than 1%. Therefore, in this study, the concentration of calcium salts was kept below 1% for each calcium salt treatment group.

3. Crude Enzyme Extraction

The crude enzyme extraction was carried out based on the method described by Pressey R & Woods FW (1992). Specifically, 2% NaCl for PG activity and 10% NaCl for PE activity, both in 150 mM sodium acetate buffer (pH 4.5), were applied to 5 g of onion samples. Homogenization of the mixture occurred at 20,000 rpm for 5 minutes using a T10 Basis homogenizer (IKA, Staufen, Germany). Following homogenization, the onion extract underwent stirring for 1 hour at 4℃ with a magnetic bar. The stirred sample was subsequently subjected to centrifugation at 4℃ and 10,000 rpm for 20 minutes using a 5430R centrifuge (Eppendorf, Hamburg, Germany), and the resulting supernatant was filtered through a 0.45 μm membrane filter. Salting-out was achieved by the addition of ammonium sulfate (99.0%) to the filtrate, followed by centrifugation at 4℃ for 30 minutes to yield a pellet. The pellet was finally dissolved in 5 mL of 150 mM sodium acetate buffer (pH 4.5) to obtain the enzyme extraction. The resulting enzyme extraction was stored at −20℃ for subsequent enzyme

activity assessments.

4. Polygalacturonase (PG) Activity

PG activity was measured spectrophotometrically based on the formation of reducing groups from polygalacturonic acid and quantifying these with 2-cyanoacetamide (Gross KC 1982). The reaction mixture (400 μL) containing 0.2% polygalacturonic acid (350 μL) and enzyme sample (50 μL) was incubated at 40℃ for 10 min. The reaction was stopped by the addition of 2 mL of cold 10 mM borate buffer, pH 9.0, and 0.4 mL 1% cyanoacetamide. The samples were mixed, immersed in an oil bath at 100℃ for 10 min and cooled immediately in ice. After equilibration at room temperature, the absorbance was measured at 276 nm and 22℃ using a spectrophotometer (Synergy HTX; Biotech Instruments., Winooski, VT, USA). Blanks were prepared in the same way but using 50 μL of 40 mM Na-acetate buffer (pH 4.4). The amount of reducing groups formed was calculated using a standard curve of mono-*d*-galacturonic acid, assuming that the concentration of this acid is proportional to the concentration of reducing groups. PG units were reported in mM mono-*d*-galacturonic acid per minute at 40℃.

5. Pectin Methylesterase (PE) Activity

PE activity was determined according to the method proposed by Simon H & Tucker GA (1999). The reaction mixture consisted of substrate solution containing 0.5% pectin in 0.05 M acetate buffer (pH 5.2) and crude enzyme (10:1, v/v), was responded at 30℃ for 60 min. This reaction mixture was maintained by addition of 200 μL of 0.5 K permanganate per mL in ice water bath for 15 min, and then added 200 μL of 0.5 M sodium arsenite and 0.6 mL distilled water in room temperature for 1 hr. This reaction mixture was sealed after addition of 2 mL of pentan-2,4-dion for developing color at 60℃ for 15 min. Absorbance was measured at 412 nm using a spectrophotometer (Synergy HTX, Biotech Instruments., Winooski, VT, USA) in room temperature. The methanol was used as standard.

6. Measurement of Calcium Ion Concentration

The calcium ion concentration of onion tissue based on calcium salt treatment and storage duration was studied by measuring ionic calcium concentration using a perfectION combination calcium electrode (Mettler-Toledo Inc., Schwerzenbach, Switzerland) combined with a pH meter. The electrical potential (mV) was linearly (R^2=0.999) correlated to the logarithm of Ca^{2+} concentration at the $CaCl_2$ concentration range of 10−40 mM. A 20 mg of the lyophilized onion tissue was added to 5 mL of distilled water and then the 0.4 mL of perfectION calcium ionic strength adjuster (ISA) containing KCl (Mettler-Toledo Inc., Schwerzenbach, Switzerland) was added into each sample solution before the measurement and two minutes were required for stable reading.

7. Measurement of Rheological Properties

The rheological properties of onion tissue based on calcium salt treatment and storage duration were measured using a rheometer (CR-100D, Sun Scientific Co., Ltd, Tokyo, Japan) under the following conditions: MODE 20, load cell: 10 kg, deformation: 30%, table speed: 60 mm/min. The samples were cut into a 1 cm^2 size. Three individually prepared samples were tested.

8. Measurement of Color Values

The color values of onion tissues onion tissue based on calcium salt treatment and storage duration were measured as lightness (L), redness (a), and yellowness (b) using a colorimeter (JS-555, Color Techno System Co., Ltd, Tokyo, Japan). The measurement was repeated at least five times to obtain an average value. The L, a, and b values of the standard white plate used were 98.35, 1.73, and −1.42, respectively.

9. Statistical Analysis

The experimental data were subjected to analysis of variance (ANOVA). Significant differences between the mean values, as determined from measurements carried out in five replicate tests (i.e., p<0.05), were obtained by Duncan's multiple-range test using statistical analysis software (SPSS 20.0, IBM Inc., NY, USA).

RESULTS AND DISCUSSION

1. Polygalacturonase (PG) Activity

Polygalacturonase (PG), also known as pectinase or pectolase, is an enzyme that hydrolyzes the glycosidic bonds in pectic acid (polygalacturonic acid) to produce D-galacturonic acid. This enzyme is known to cause the softening of plant cell tissues by breaking down the glucosidic linkages in

pectin, thereby increasing the solubility of the substances. Pressey noted that fruits which do not soften lack polygalacturonase activity, and that the absence of polygalacturonase prevents the softening process (Pressey R & Avants JK 1971). The PG activity of onion tissue treated with various calcium salts for storage periods are presented in Fig. 1. Regarding the activity of PG in onion tissues during storage, the addition of calcium carbonate at concentrations of 30, 50, and 90 mM showed an initial increase followed by a decrease in PG activity as storage time increased. However, the 70 mM treatment group exhibited a continuous decrease in PG activity (p<0.05). After 60 days of storage, the lowest PG activity was observed in the 70 mM treatment group, with an activity level of 12.10 units, compared to 53.52 units in the control group (fresh

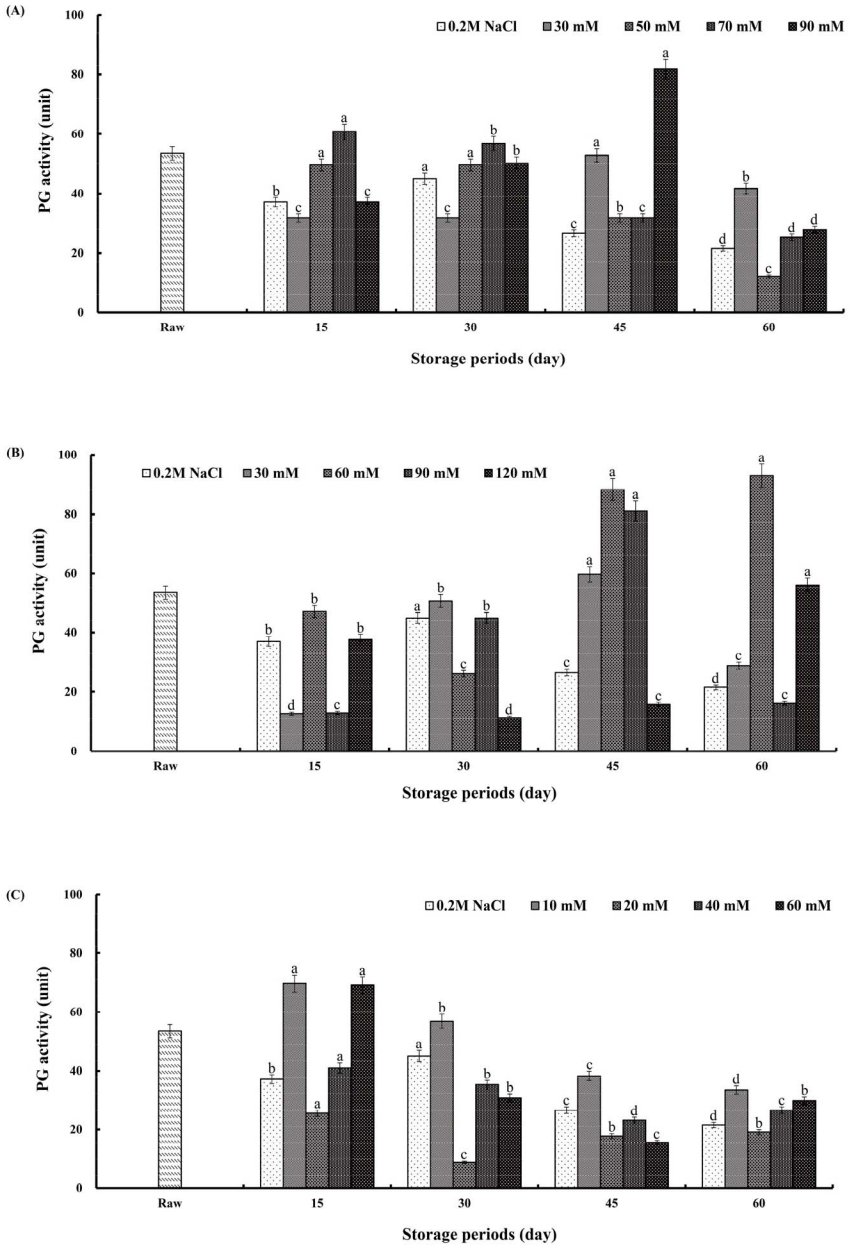

Fig. 1. The polygalacturonase activity of onion tissue treated with various calcium salts for storage period.
(A) Calcium carbonate addition group. (B) Calcium hydroxide addition group. (C) Calcium chloride addition group.
Data represent the mean values for each sample±standard deviation (n=5).
$^{a\sim d}$ Means with different superscripts on the graph are significantly different (p<0.05).

onions), indicating about a 4.4-fold inhibition of PG activity. For the calcium hydroxide addition group, the 0.2M NaCl, 30, 60, and 90 mM treatment groups initially showed an increase followed by a decrease in PG activity as storage time increased, whereas the 120 mM treatment group showed a decrease followed by an increase ($p<0.05$). After 60 days of storage, the lowest PG activity was observed in the 90 mM treatment group, with an activity level of 16.19 units, compared to 53.52 units in the control group (fresh onions), indicating about a 3.3-fold inhibition of PG activity. In the calcium chloride addition group, the 10 mM treatment group showed a decrease in PG activity as storage time increased, while the 20, 40, and 60 mM treatment groups exhibited a decrease followed by an increase in PG activity ($p<0.05$). After 60 days of storage, the lowest PG activity was observed in the 20 mM treatment group, with an activity level of 19.15 units, compared to 53.52 units in the control group (fresh onions), indicating about a 2.8-fold inhibition of PG activity. This is supported by previous research that demonstrated a negative correlation between calcium content and polygalacturonase activity in Sauvignon Blanc and Semillon grapes (Cabanne C & Doneche B 2001). Additionally, calcium may enhance the resistance of plant cell walls to polygalacturonases by inhibiting the enzyme's interaction with the pectin polymer (Pagel W & Heitefuss R 1990). Calcium can crosslink unesterified regions of pectin, reducing the size of pores in the plant cell wall and subsequently restricting the diffusion of polygalacturonases through the wall (Glenn GM *et al* 1988; Carpita NC & Gibeaut DM 1993). Therefore, the most effective concentration for inhibiting PG activity and preventing the softening of onion tissues during storage was found to be the 70 mM calcium carbonate treatment group.

2. Pectin Methylesterase (PE) Activity

Fruit softening is accompanied by the solubilization and depolymerization of pectin, due to the changes in activities of PG and PE. The PE could strengthen the interaction between carboxylic acid and calcium, due to PE catalyzed pectin homogalacturonan de-esterification, which could stabilize pectin (Zhang LF *et al* 2019). The PE activity of onion tissue treated with various calcium salts for storage periods are presented in Fig. 2. In the calcium carbonate addition group, the 50, 70, and 90 mM treatment groups initially showed a decrease in PE activity, followed by an increase as the storage period progressed. However, compared to the control group treated with 0.2M NaCl, the PE activity was not significantly higher, with the highest activity observed on 15 days in the 90 mM treatment group. In the calcium hydroxide addition group, no distinct pattern of change in PE activity was observed across all concentration treatment groups throughout the storage period, and PE activity remained lower than that of the control group treated with 0.2M NaCl across all concentrations and storage periods. For the calcium chloride addition group, the 20, 40, and 60 mM treatment groups showed an increase in PE activity followed by a decrease as the storage period increased, with higher activity observed on 30 and 45 days. However, the 10 mM treatment group exhibited a continuous decrease in PE activity over time. Additionally, on 30 days of storage, all concentration treatment groups showed higher PE activity compared to the control group treated with 0.2M NaCl, with the 20 mM treatment group displaying the highest PE activity at 87.15 units. PE catalyzes the de-esterification of pectin by removing methyl groups to produce free carboxyl groups, which, in calcium-deficient conditions, become substrates for pectin-depolymerizing enzymes like polygalacturonases and pectate lyase, leading to texture degradation (Sila DN *et al* 2009). This result is reasonable, as the methyl groups in pectin molecules inhibit the formation of calcium bridges between them (Thakur BR *et al* 1997). PME facilitates the demethoxylation of homogalacturonan in the plant cell wall, releasing methanol and protons, which in turn create negatively charged carboxyl groups (Sila DN *et al* 2009). Additionally, Sirijariyawat *et al* (2012) found that the microstructure of a sample infused with PME and calcium closely resembled that of fresh mango flesh. Therefore, calcium chloride at a concentration of 20 mM was identified as the most effective calcium salt for enhancing PE activity. Consequently, treating onion tissues with 20 mM calcium chloride is expected to effectively inhibit the softening process.

3. Measurement of Calcium Ion Concentration

The concentration of calcium ions in onion tissues treated with various calcium salts for storage periods is presented in Fig. 3. In the calcium carbonate addition group, the 30 mM treatment groups showed a trend of decreasing calcium ion concentration followed by an increase as the storage period progressed, while the 50 mM treatment group exhibited no significant changes in calcium ion concentration during the

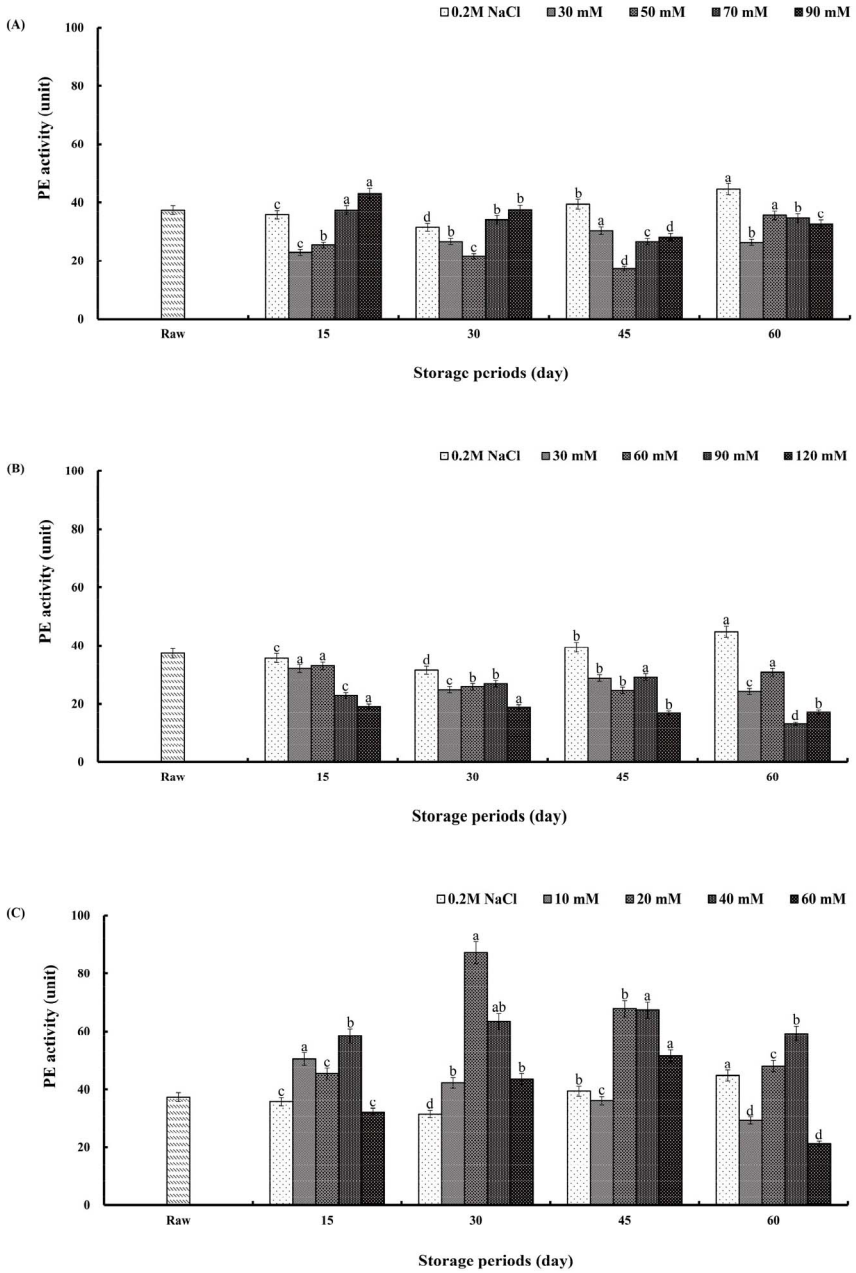

Fig. 2. The pectin methylesterase activity of onion tissue treated with various calcium salts for storage period.
(A) Calcium carbonate addition group. (B) Calcium hydroxide addition group. (C) Calcium chloride addition group.
Data represent the mean values for each sample±standard deviation (n=5).
$^{a\sim d}$ Means with different superscripts on the graph are significantly different ($p<0.05$).

storage period. The 70 mM treatment group showed an increase in calcium ion concentration until 30 days of storage, after which the concentration remained stable, whereas the 90 mM treatment group maintained a stable concentration until 45 days of storage, followed by a sharp increase at 60 days of storage. Among the calcium carbonate addition groups, the 90 mM treatment at 60 days of storage showed the highest calcium ion concentration of 1.639 mM. In the calcium hydroxide addition group, all treatment concentrations resulted in an increase in calcium ion concentration in onion tissues as the storage period increased, with the 120 mM treatment group showing the highest concentration of 22.785 mM at 45 days of storage. For the calcium chloride addition group, the 10, 20, and 40 mM treatment group maintained stable calcium ion

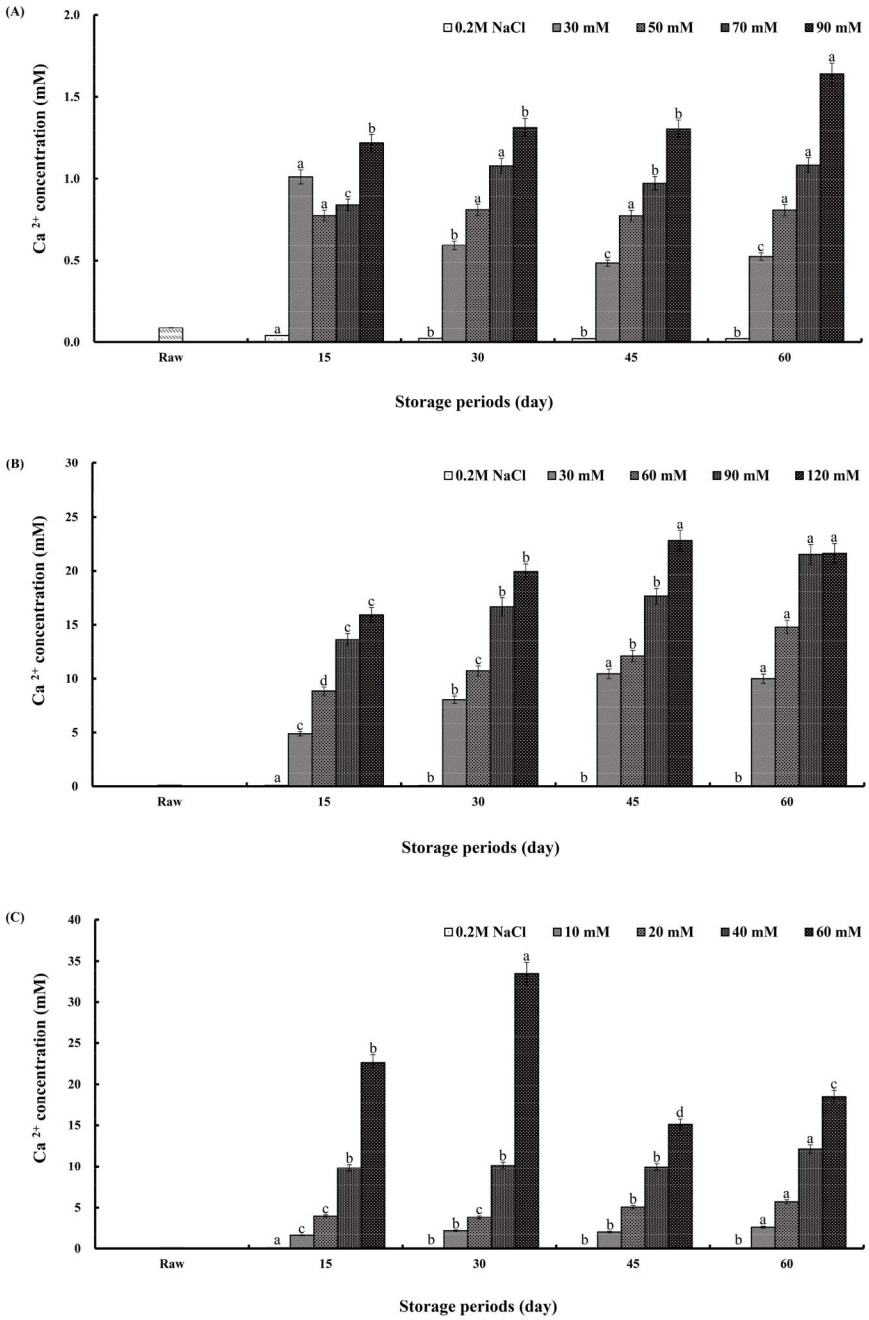

Fig. 3. The concentration of calcium ions in onion tissues treated with various calcium salts for storage periods.
(A) Calcium carbonate addition group. (B) Calcium hydroxide addition group. (C) Calcium chloride addition group.
Data represent the mean values for each sample±standard deviation (n=5).
$^{a\sim d}$ Means with different superscripts on the graph are significantly different (p<0.05).

concentrations until 45 days, followed by a sharp increase at 60 days of storage, while the 60 mM treatment group showed the highest concentration of 33.435 mM at 30 days of storage. In this regard, Greenward I (1938) reported that the solubility of calcium ions is affected by both the type of anion bound to calcium and the pH of the calcium salt solution, and the stability of calcium ions can vary depending on the presence of organic acids. These findings indicate that the penetration of calcium ions into onion tissues varies depending on the type of calcium salt used, with calcium chloride demonstrating the most effective penetration rate across the storage period when considering the different concentrations of calcium salts.

4. Measurement of Rheological Properties

The change of strength and hardness in onion tissues treated with various calcium salts for storage periods is presented in Figs. 4 and 5. In the results of the strength measurement, in the calcium carbonate addition group, increasing the calcium salt concentration resulted in a rise in strength up to 30 days of storage, followed by a rapid decline after 45 days of storage. The highest strength was observed on 30 days in the 90 mM treatment group, measuring 18,180 g/cm². In the calcium hydroxide addition group, the 30, 90, and 120 mM

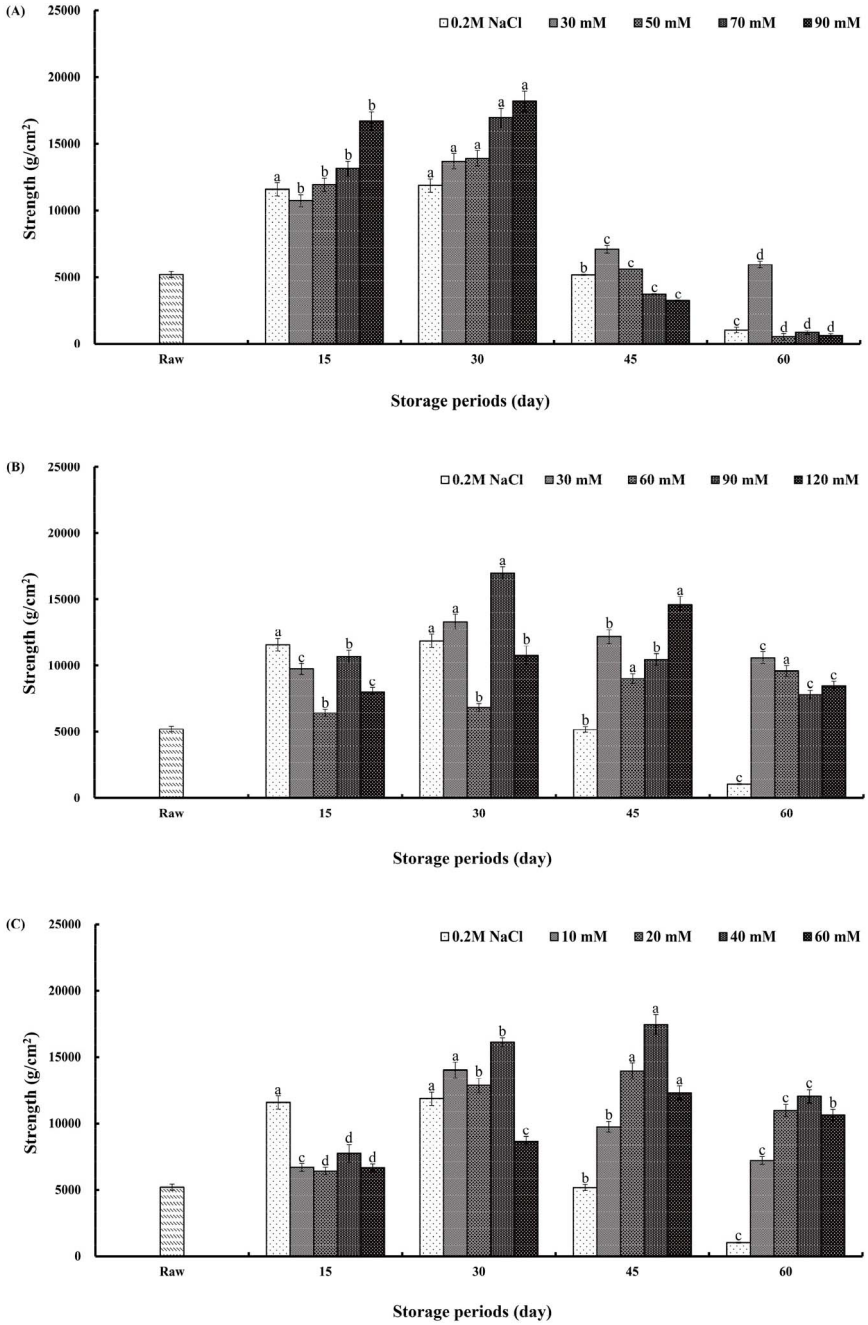

Fig. 4. The change of strength in onion tissues treated with various calcium salts for storage periods. (A) Calcium carbonate addition group. (B) Calcium hydroxide addition group. (C) Calcium chloride addition group. Data represent the mean values for each sample±standard deviation (n=5).
[a~d] Means with different superscripts on the graph are significantly different (p<0.05).

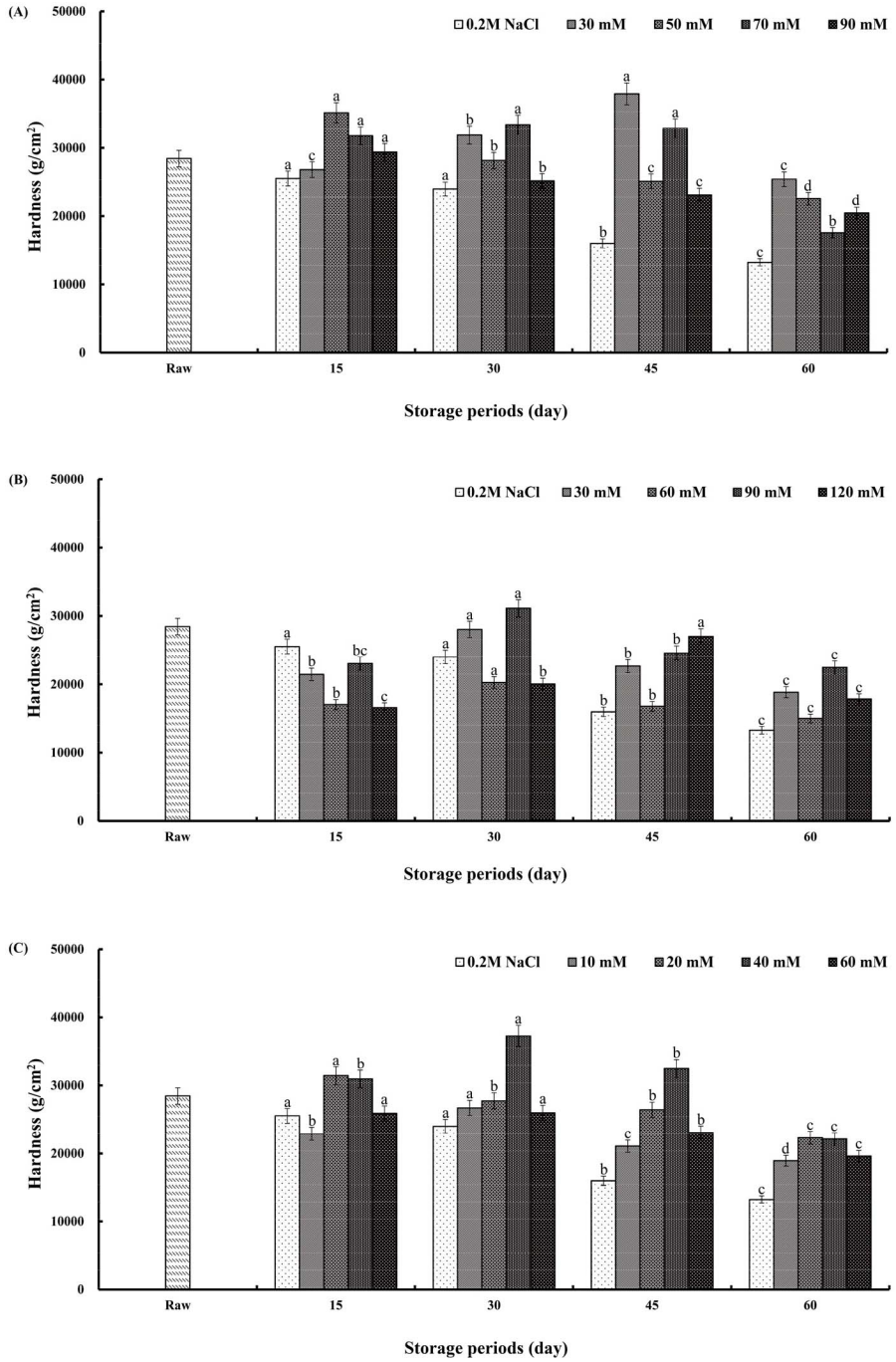

Fig. 5. The change of hardness in onion tissues treated with various calcium salts for storage periods.
(A) Calcium carbonate addition group. (B) Calcium hydroxide addition group. (C) Calcium chloride addition group. Data represent the mean values for each sample±standard deviation (n=5).
[a~d] Means with different superscripts on the graph are significantly different (p<0.05).

treatments initially showed an increase in strength, which then decreased as the storage period extended. In contrast, the 60 mM treatment group exhibited a gradual increase in strength up to 60 days of storage. The highest strength was observed on 30 days of storage in the 90 mM treatment group, with a value of 16,993 g/cm². In the calcium chloride treatment group, all concentrations demonstrated an initial increase in strength over time, followed by a subsequent decline. The

highest strength was observed on 45 days of storage in the 40mM treatment group, with a value of 17,450 g/cm². Thus, among the different calcium salt concentrations, the calcium chloride treatment consistently maintained the highest strength over the extended storage period. In the results of the hardness measurement, the calcium carbonate treatment groups at 30 mM and 70 mM was observed an initial increase in hardness, which later declined as the storage period extended. In contrast, the 50 mM and 90 mM treatment groups exhibited a consistent decrease in hardness throughout the storage period. Similarly, the control group treated with 0.2M NaCl also was observed a decrease in hardness as storage time increased. The highest firmness was observed in the 30 mM treatment group, reaching 37,920 g/cm² on 45 days of storage. In the calcium hydroxide treatment group, all concentrations initially were observed an increase in hardness, followed by a decline as the storage period progressed. On 15 days of storage, the hardness of all treatment groups was lower than that of the control group treated with 0.2M NaCl. The highest hardness was observed in the 90 mM treatment group, with a value of 31,116 g/cm² on 30 days of storage. Compared to the hardness of fresh onions, the addition of calcium hydroxide did not increase the hardness of onion tissues. In the calcium chloride treatment group, the 10 mM and 40 mM treatment groups was observed an initial increase in hardness, followed by a decline as storage time increased, while the 50 mM and 90 mM treatment groups consistently was observed a decrease in hardness throughout the storage period. The highest hardness was observed in the 40 mM treatment group, reaching 37,263 g/cm² on 30 days of storage. When calcium is present in cell walls, they have increased strength, which means the plant cells are able to better resist damage (Dodgson J *et al* 2023). And treatment of fruits with calcium may postpone senescence by maintaining membrane integrity and increasing firmness of cell wall (Ishaq S *et al* 2009; Zhi HH *et al* 2017). Thus, when considering hardness over the storage period, calcium chloride was found to be the most effective calcium salt among the concentrations tested.

5. Measurement of Color Values

The change of color parameters in onion tissues treated with various calcium salts for storage periods is presented in Tables 1, 2 and 3. For the L* value, the control group treated with 0.2M NaCl demonstrated a decrease from 84.33 to 13.81

Table 1. The changes of L* value in onion tissues treated with various calcium salts for storage periods

Color parameter	Samples		Storage periods (day)			
			15	30	45	60
L*	Control (0.2M NaCl)		84.33±9.49[1)a2)]	23.13±2.60^{b}	18.41±2.08^{b}	13.81±1.56^{b}
	Calcium carbonate	30 mM	87.53±9.85^{a}	25.50±2.87^{b}	19.67±2.22^{b}	16.58±1.87^{b}
		50 mM	82.41±9.28^{a}	24.59±2.77^{b}	23.09±2.60^{b}	17.76±2.00^{b}
		70 mM	88.48±9.96^{a}	22.75±2.56^{b}	21.36±2.40^{b}	16.60±1.87^{b}
		90 mM	84.83±9.56^{a}	20.96±2.36^{b}	24.28±2.73^{b}	18.03±2.03^{b}
	Calcium hydroxide	30 mM	16.90±1.90^{b}	10.64±1.20^{c}	22.87±2.57^{a}	24.49±2.76^{a}
		60 mM	17.63±1.98^{a}	8.01±0.90^{b}	18.98±2.14^{a}	20.94±2.36^{a}
		90 mM	14.85±1.67^{b}	11.08±1.25^{c}	19.58±2.21^{a}	18.12±2.04^{ab}
		120 mM	15.48±1.74^{c}	8.94±1.01^{d}	19.74±2.22^{b}	23.75±2.67^{a}
	Calcium chloride	10 mM	17.84±2.01^{b}	18.80±2.12^{b}	15.77±1.78^{b}	23.85±2.69^{a}
		20 mM	17.77±2.00^{b}	17.15±1.93^{b}	16.94±1.91^{b}	22.70±2.56^{a}
		40 mM	17.90±2.02^{b}	15.46±1.74^{b}	16.66±1.88^{b}	22.77±2.57^{a}
		60 mM	17.59±1.98^{b}	17.08±1.92^{b}	17.88±2.01^{b}	22.73±2.56^{a}

[1)] Data represent the mean values for each sample±standard deviation (n=5).
[2)] $^{a\sim d}$ Means with different superscripts on the graph are significantly different (p<0.05)

Table 2. The changes of a* value in onion tissues treated with various calcium salts for storage periods

Color parameter	Samples		Storage periods (day)			
			15	30	45	60
a*	Control (0.2M NaCl)		−12.07±1.36[1)c2)]	0.85±0.10[a]	−6.00±0.68[b]	0.92±0.11[a]
	Calcium carbonate	30 mM	−12.38±1.40[c]	2.90±0.33[a]	−3.50±0.40[b]	2.27±0.26[a]
		50 mM	−12.37±1.39[d]	3.74±0.43[a]	−5.18±0.59[c]	−0.06±0.01[b]
		70 mM	−8.81±1.00[c]	3.22±0.36[a]	−8.46±0.96[c]	0.39±0.05[b]
		90 mM	−10.23±1.15[c]	2.93±0.33[a]	−6.60±0.74[b]	2.50±0.28[a]
	Calcium hydroxide	30 mM	−12.65±1.43[b]	−11.69±1.32[b]	0.77±0.09[a]	0.58±0.07[a]
		60 mM	−7.75±0.87[b]	−10.31±1.16[c]	0.78±0.09[a]	0.19±0.02[a]
		90 mM	−9.54±1.08[b]	−13.86±1.56[c]	0.79±0.09[a]	0.40±0.05[a]
		120 mM	−11.50±1.30[c]	−10.35±1.17[c]	0.94±0.11[b]	3.14±0.35[a]
	Calcium chloride	10 mM	−6.86±0.78[b]	−8.16±0.92[bc]	−8.65±0.97[c]	−0.27±0.03[a]
		20 mM	−7.51±0.85[b]	−9.53±1.08[c]	−7.03±0.79[b]	0.02±0.00[a]
		40 mM	−6.94±0.78[b]	−6.38±0.72[b]	−8.59±0.97[c]	−0.32±0.04[a]
		60 mM	−7.58±0.86[b]	−6.82±0.77[b]	−9.25±1.05[c]	0.46±0.05[a]

[1)] Data represent the mean values for each sample±standard deviation (n=5).
[2)] [a~d] Means with different superscripts on the graph are significantly different (p<0.05).

Table 3. The changes of b* value in onion tissues treated with various calcium salts for storage periods

Color parameter	Samples		Storage periods (day)			
			15	30	45	60
b*	Control (0.2M NaCl)		15.10±1.70[1)a2)]	1.62±0.19[b]	−0.48±0.06[c]	1.18±0.14[b]
	Calcium carbonate	30 mM	22.20±2.50[a]	4.90±0.55[b]	5.29±0.60[b]	4.88±0.55[b]
		50 mM	22.39±2.52[a]	5.71±0.65[b]	5.56±0.63[b]	4.95±0.56[b]
		70 mM	24.57±2.77[a]	5.96±0.68[b]	5.55±0.63[b]	5.21±0.59[b]
		90 mM	21.91±2.47[a]	4.83±0.55[b]	4.06±0.46[b]	6.27±0.71[b]
	Calcium hydroxide	30 mM	7.77±0.88[a]	−10.80±1.22[c]	5.49±0.62[b]	4.88±0.55[b]
		60 mM	5.80±0.66[c]	−12.94±1.46[d]	8.40±0.95[b]	12.11±1.36[a]
		90 mM	8.30±0.94[a]	−8.82±1.00[b]	10.05±1.13[a]	10.02±1.13[a]
		120 mM	8.32±0.94[b]	−11.93±1.35[c]	7.52±0.85[b]	15.10±1.70[a]
	Calcium chloride	10 mM	2.63±0.30[b]	2.32±0.26[b]	3.59±0.41[a]	1.31±0.15[c]
		20 mM	3.19±0.36[a]	3.45±0.39[a]	2.17±0.25[b]	0.71±0.08[c]
		40 mM	3.26±0.37[a]	2.79±0.32[a]	2.76±0.32[a]	0.15±0.02[b]
		60 mM	2.52±0.29[b]	2.13±0.24[b]	3.50±0.39[a]	0.77±0.09[c]

[1)] Data represent the mean values for each sample±standard deviation (n=5).
[2)] [a~d] Means with different superscripts on the graph are significantly different (p<0.05).

as the storage period increased. In the calcium carbonate addition groups, the L* value decreased consistently over time, regardless of the calcium salt concentration. In contrast, the calcium chloride addition groups maintained similar L* values until 45 days of storage, after which they slightly increased by 60 days, from 22.70 to 23.85. The calcium hydroxide addition groups did not show a clear trend in L* values with respect to either calcium salt concentration or storage period. Notably, the calcium carbonate addition groups showed the most similar L* value changes to the control group, with the 90 mM treatment group maintaining the highest L* value up to 60 days of storage.

As for the a* value, the control group treated with 0.2M NaCl displayed fluctuations, with repeated increases and decreases throughout the storage period. The calcium carbonate addition group exhibited a similar pattern of a* value changes as the control group over time. In the calcium hydroxide addition group, the a* value peaked on 45 days of storage. In contrast, the calcium chloride addition group did not show a clear trend in a* values in relation to either calcium salt concentration or storage period.

Regarding the b* value, the control group treated with 0.2 M NaCl showed a sharp decrease as the storage period progressed, followed by a slight increase on 60 days of storage. In the calcium carbonate addition group, the b* value peaked on 15 days of storage, followed by a decrease from 30 to 60 days. The calcium hydroxide addition group showed a sharp decrease in b* value on 30 days, followed by an increase. Among all groups, the calcium chloride addition group exhibited the lowest b* values over the storage period compared to the control, calcium carbonate, and calcium hydroxide addition groups. None of the calcium salt addition groups displayed a pattern of b* value changes similar to the control group treated with 0.2M NaCl. The results of the color in onion tissues treated with various calcium salts for storage periods showed that the treatments used had no detrimental effect on the color compared to the control treatment. Similar to the results of the current study, Moradinezhad F *et al* (2019) showed that the post-harvest application of calcium salts has no significant effect on the color of jujube fruit. Hernández-Muñoz P *et al* (2006) reported higher lightness (L*) in strawberries dipped in calcium solution, while they did not observe any effect of calcium dipping on the fruit hue angle. In addition, calcium salts, especially calcium lactate and calcium chloride, are used in combination with browning inhibitors as firmness agents in a wide variety of whole, peeled, and fresh-cut fruit and vegetables (Raybaudi-Massilia RM *et al* 2007).

CONCLUSIONS

The objective of this study was to investigate the effects of different calcium salts (calcium carbonate, calcium hydroxide, and calcium chloride) on the softening of onion tissues during storage. The study focuses on the enzymatic activities of polygalacturonase (PG) and pectin methylesterase (PE), calcium ion concentration, tissue strength, hardness, and color changes over varying storage periods. The findings suggest that calcium salts, particularly calcium chloride at specific concentrations, can effectively inhibit the softening of onion tissues during storage by influencing enzymatic activities, maintaining calcium ion concentrations, and preserving tissue strength and hardness. The most effective treatments identified were 70 mM calcium carbonate for inhibiting PG activity and 20 mM calcium chloride for enhancing PE activity, with calcium chloride also performing well in maintaining tissue strength, hardness, and color stability. These insights can guide the use of calcium salts in food preservation to extend the shelf life and quality of fresh produce.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

Aghdam MS, Hassanpouraghdam MB, Paliyath G, Farmani B (2012) The language of calcium in postharvest life of fruits, vegetables and flowers. Sci Hortic 144: 102-115.

Albishi T, John JA, Al-Khalifa AS, Shahidi F (2013) Phenolic content and antioxidant activities of selected potato varieties and their processing by-products. J Funct Foods 5(2): 590-600.

Alzamora SM, Salvatori D, Tapia MS, López-Malo A, Welti-Chanes J, Fito P (2005) Novel functional foods from vegetable matrices impregnated with biologically active compounds. J Food Eng 67(1-2): 205-214.

Ashraf M, Harris PJC (2004) Potential biochemical indicators of salinity tolerance in plants. Plant Sci 166(1): 3-16.

Brant LA (2003) Calcium the Essential Mineral. Formulation and Ingredient Challenges, Issue November. https://www.preparedfoods.com (accessed on 24. 9. 2024).

Cabanne C, Doneche B (2001) Changes in polygalacturonase activity and calcium content during ripening of grape berries. Am J Enol Vitic 52(4): 331-335.

Carpita NC, Gibeaut DM (1993) Structural models of primary cell walls in flowering plants: Consistency of molecular structure with the physical properties of the walls during growth. Plant J 3(1): 1-30.

Dodgson J, Weston AK, Marks DJ (2023) Tomato firmness and shelf-life increased by application of stimulated calcium. Crops 3(4): 251-265.

Figueroa CR, Opazo MC, Vera P, Arriagada O, Díaz M, Moya-León MA (2012) Effect of postharvest treatment of calcium and auxin on cell wall composition and expression of cell wall-modifying genes in the Chilean strawberry (*Fragaria chiloensis*) fruit. Food Chem 132(4): 2014-2022.

Gao Q, Xiong T, Li X, Chen W, Zhu X (2019) Calcium and calcium sensors in fruit development and ripening. Sci Hortic 253: 412-421.

Glenn GM, Reddy ASN, Poovaiah BW (1988) Effect of calcium on cell wall structure, protein phosphorylation and protein profile in senescing apples. Plant Cell Physiol 29(4): 565-572.

González-Peña D, Dudzik D, Colina-Coca C, Ancos BD, García A, Barbas C, Sánchez-Moreno C (2015) Evaluation of onion as a functional ingredient in the prevention of metabolic impairments associated to diet-induced hypercholesterolaemia using a multiplatform approach based on LC-MS, CE-MS and GC-MS. J Funct Foods 19: 363-375.

Goudra GP, Ramachandra CT, Udaykumar N, Sharanagouda H (2018). Studies on drying characteristics of onion (variety-*Arka kalyan*) slices using different drying methods. J Pharmacogn Phytochem 7(2): 1013-1016.

Greenwald I (1938) The dissociation of some calcium salts. J Biol Chem 124(2): 437-452.

Gross KC (1982) A rapid and sensitive spectrophotometric method for assaying polygalacturonase using 2-cyanoacetamide. HortSciences 17(6): 933-934.

Hernández-Muñoz P, Almenar E, Ocio MJ, Gavara R (2006) Effect of calcium dips and chitosan coatings on postharvest life of strawberries (*Fragaria* x *ananassa*). Postharvest Biol Technol 39(3): 247-253.

Hocking B, Tyerman SD, Burton RA, Gilliham M (2016) Fruit calcium: Transport and physiology. Front Plant Sci 7: 569.

Hussein JB, Soji MY, Abiona OO, Oke MO (2018) Drying characteristics of osmotically pretreated red onion slices via hot air oven. J Food Process Technol 9(5): 1000733.

Ishaq S, Rathrore HRA, Masud T, Ali S (2009) Influence of post-harvest calcium chloride application, ethylene absorbent and modified atmosphere on quality characteristics and shelf-life of apricot (*Prunus armeniaca* L.) fruit during storage. Pak J Nutr 8(6): 861-865.

Khan MKI, Ansar M, Nazir A, Maan AA (2016) Sustainable dehydration of onion slices through novel microwave hydro-diffusion gravity technique. Innov Food Sci Emerg Technol 33: 327-332.

Lötze E, Joubert J, Theron KI (2008) Evaluating pre-harvest foliar calcium applications to increase fruit calcium and reduce bitter pit in 'Golden Delicious' apples. Sci Hortic 116(3): 299-304.

Luna-Guzmán I, Barrett DM (2000) Comparison of calcium chloride and calcium lactate effectiveness in maintaining shelf stability and quality of fresh-cut cantaloupes. Postharvest Biol Technol 19(1): 61-72.

Mahajan BVC, Dhatt A (2004) Studies on postharvest calcium chloride application on storage behaviour and quality of Asian pear during cold storage. J Food Agric Environ 2(3-4): 157-159.

Moradinezhad, F, Ghesmati M, Khayyat M (2019) Postharvest calcium salt treatment of fresh jujube fruit and its effects on biochemical characteristics and quality after cold storage. J Hort Res 27(2): 39-46.

Pagel W, Heitefuss R (1990) Enzyme activites in soft rot pathogenesis of potato tubers: Effects of calcium, pH, and degree of pectin esterfication on the activities of polygalacturonase and pectate lyase. Physiol Mol Plant Pathol 37(1): 9-25.

Pitzschke A, Xue H, Persak H, Datta S, Seifert GJ (2016) Post-translational modification and secretion of azelaic

acid induced 1 (AZI1), a hybrid proline-rich protein from arabidopsis. Int J Mol Sci 17(1): 85.

Pressey R, Woods FW (1992) Purification and properties of two pectinesterases from tomatoes. Phytochem 31(4): 1139-1142.

Pressey R, Avants JK (1971) Effect of substrate size on the activity of tomato polygalacturonase. J Food Sci 36(3): 486-489.

Raybaudi-Massilia RM, Mosqueda-Melgar J, Sobrino-López A, Soliva-Fortuny R, Martín-Belloso O (2007) Shelf-life extension of fresh-cut “Fuji” apples at different ripeness stages using natural substances. Postharvest Biol Technol 45(2): 265-275.

Shannon MC, Grieve CM (1999) Tolerance of vegetable crops to salinity. Sci Hortic 78(1-4): 5-38.

Sila DN, Van Buggenhout S, Duvetter T, Fraeye I, De Roeck A, Van Loey A, Hendrickx M (2009) Pectins in processed fruits and vegetables: Part II－structure-function relationships. Compr Rev Food Sci Food Saf 8(2): 86-104.

Simons H, Tucker GA (1999) Simultaneous co-suppression of polygalacturonase and pectinesterase in tomato fruit: Inheritance and effect on isoform profiles. Phytochem 52(6): 1017-1022.

Sirijariyawat A, Charoenrein S, Barrett DM (2012) Texture improvement of fresh and frozen mangoes with PME and calcium infusion. J Sci Food Agric 92(13): 2581-2586.

Thakur BR, Singh RK, Handa AK, Rao MA (1997) Chemistry and uses of pectin-a review. Crit Rev Food Sci Nutr 37(1): 47-73.

Wang D, Yeats TH, Uluisik S, Rose J, Seymour GB (2018) Fruit softening: Revisiting the role of pectin. Trends Plant Sci 23(4): 302-310.

Zhang LF, Wang P, Chen FS, Lai SJ, Yu HG, Yang HS (2019) Effects of calcium and pectin methylesterase on quality attributes and pectin morphology of jujube fruit under vacuum impregnation during storage. Food Chem 289: 40-48.

Zhang Z, Xing Y, Ramakrishnan M, Chen C, Xie F, Hua Q, Chen J, Zhang R, Zhao J, Hu G, Qin Y (2022) Transcriptomics-based identification and characterization of genes related to sugar metabolism in ‘hongshuijing’ pitaya. Hortic Plant J 8(4): 450-460.

Zhi HH, Liu QQ, Dong Y, Liu MP, Zong W (2017) Effect of calcium dissolved in slightly acidic electrolyzed water on antioxidant system, calcium distribution, and cell wall metabolism of peach in relation to fruit browning. J Hortic Sci Biotechnol 92(6): 621-629.

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