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# Ethylene Biosynthesis Inhibition and Metabolic Response of Apple 'Delbar Estival' under 1-MCP and cacl$_2$ Treatments for Keeping Quality During Storage

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**ABSTRACT**

*Fruit tissue softening and quality loss are among the main limiting factors in storage life of fruits resulting from an increase in transpiration and mechanical damage during transportation. Further, exposure to ethylene is the reason for the decline the quality and storage life of fruits. Ethylene action inhibitors such as 1-methylceyclopropene (1-MCP), and also calcium chloride ($CaCl_2$) treatments can improve fruit quality through ethylene inhibition and delaying fruit ripening. A split plot factorial experiment based on completely randomized design with three replications was performed to study the effect of 1-MCP and $CaCl_2$ on postharvest quality changes in apple 'Delbar estival'. The main plot was storage time at four different times with 2 months intervals (60, 120, 180, and 240 days), sub-plot included 1-MCP (0, 0.001, and 0.005 $\mu L.L^{-1}$) and CaCl2 (0, 1, and 2 %). Results indicated that fruit acidity was decreased significantly as storage time increased. 1-MCP and $CaCl_2$ treatments, significantly increased fruit firmness, soluble solids content (SSC), vitamin C content, and suppressed ethylene production during storage. Taken together, results indicated that 1-MCP and $CaCl_2$ treatments significantly increased storage life of apple 'Delbar estival' and decreased the biochemical changes rate during storage, resulted in higher quality of stored fruits.*

**Key words:** *1-MCP, calcium chloride, Delbar Estival, shelf life, vitamin C*

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## INTRODUCTION

Apple (Malus domestica Borkh.) belongs to Rosaceae family and is one of the most important fruits in temperate zones. Apple fruit plays an important role in human health due to the high content of polyphenols (Yuri et al., 2012). The total content of apple production in the world is about 77.5 million tons. Iran produces 1.7 million tons annually and is the rank 8 of the largest producers in the world (FAO, 2012).

Quality indices of fruits is a key factor in retails and markets of countries with high quality production (Konopacka and Plocharsski, 2006). Quality of apple depends on many factors including, cultivar, nutrition, fruit ripening at harvest, and storage condition (Varela et al., 2007). Harvested apples are biologic systems with active metabolism and have a high risk of corruption, hence finding appropriate methods for keeping quality during storage is a determining factor in marketability. 1-MCP has been added to the list of treatments to extend fruits storage life (Burns, 2008). A number of studies have revealed that application of 1-MCP can positively affect postharvest life of fruits (Selvarajah et al., 2001; Yang et al., 2011). 1-MCP actively inhibits ethylene action, delays transpiration increase, and result in keeping quality of crops. 1-MCP is the best gaseous material in order to keep quality of apple fruits, which binds to ethylene receptors and inhibits ethylene action (Watkins, 2006; Tatsuki et al., 2007). The susceptibility of fruit tissue to exogenous ethylene depends on the number and type of receptors. While the regeneration rate of ethylene receptors is higher, fruit tissue sensitivity to ethylene in the environment increases and under these condition, 1-MCP role is more efficient in ethylene suppression. It should be noted that the permeability of the fruit tissue may affect 1-MCP effectiveness (Peter and Toivonen, 2008).

Macro and micro nutrients affects fruits quality differently. Calcium (Ca) is the most important element in fruits quality. Application of Ca increased storage life in pear (Dhatt et al., 2005), peach (Manganaris et al., 2007), and strawberry (Hernandez et al., 2008). Calcium delayed senescence, ripening, and increased resistant to pathogens, also decreased frost damage in fruits through delaying cell wall senescence and increasing membrane stability (Sharpels and Johnson, 1977). Calcium regulates many metabolic processes including permeability of cell membranes, cell division, microtubules movements, and ion leakage (Hussain et al., 2012).

Li et al. (2011) reported that post-harvest treatment of 1-MCP ($1\mu L.L^{-1}$) reduced poly-galacturonase enzyme activity in mature green Ziziphus jujuba fruit tissue and thus delayed the softening of the fruits. In another study on papaya fruit, 1-MCP decreased activity of the polygalacturonase enzyme, a pectin methylesterase enzyme and resulted in decreased fruit softening, subsequently increased storage life (Ergun et al., 2006). Additionally, post-harvest application of calcium chloride reduced fruit decay, storage life disorders, transpiration, and ethylene production in apricot cv. Habi (Ali et al., 2013). Buda and Joyce (2003) reported that application of 1-MCP reduced transpiration and prevented ascorbic acid hydrolysis in pineapple. Aguayo et al. (2006) demonstrated that the combined treatment of 1-MCP and $CaCl_2$, decreased fruit softening, microbial growth, acidity, and maintained quality indices of strawberry. Overall, 1-MCP and calcium have been widely used to improve or maintain textural attributes of fruits. Considering the importance of apple fruit production in Iran, this study was performed to evaluate the effect of 1-MCP and $CaCl_2$ on keeping quality indices of apple 'Delbar estival'.

## 2.MATERIAL AND METHODS

An experiment was conducted in split plot factorial (main plot was storage time: 60, 120, 180, and 240 days after storage (2 months interval); and subplot was 1-MCP (0, 0.001, and 0.005 $\mu L.L^{-1}$) and $CaCl_2$ (0, 1, and 2%), based on completely randomized design with three replications to evaluate the effect of 1-MCP and $CaCl_2$ on extending storage life and quality attributes of apple 'Delbar estival' during storage. Fruits were exposured to 1-MCP for 19 hours. The treatment with $CaCl_2$ (0, 1, and 2%) was done through immersion of fruits in supplied concentration for 10 minutes. Additionally, extra control treatment ($2^{nd}$ control) with distilled water was applied. Fruits were immersed in $CaCl_2$ after exposure to 1-MCP, afterwards fruits were transferred to cold storage (Temp: 0±1, RH: 90-95 %).

### 2.1. Fruit firmness

Fruit firmness was determined by peeling the fruit at two equatorial sites and measuring firmness by means of a Fruit Firmness Tester equipped with an 8mm plunger tip, using five fruits from each treatment. Values were expressed in Newton.

### 2.2. Soluble solids content (SSC)

The SSC was determined based on Hernandez-Munoz et al. (2008) with some modifications. Briefly, the fruits extract for each replication was supplied. The SSC was measured using a digital refractometer (Atago Co. Ltd., Tokyo; Japan) at 20°C and expressed as Brix degree.

### 2.3. Titratable acidity (TA)

The titratable acidity was measured based on Ranganna (2003). Fruits extract was supplied and extract was titrated using NaOH (0.1 N). The volume of consumed NaOH for measuring TA was obtained based on citric acid. The following formula was used for calculating total acidity:

$A = (S.N.E.F)/C \times 100$

A: The content of organic acid in extract ($g.100\ ml^{-1}$)
S: The consumed NaOH volume (ml)
E: Equivalent of acid (citric acid in apple)
C: The volume of fruit extract (ml)

### 2.4. Determination of ethylene

The ethylene content was measured using GC (Shimadzu; USA) and following formula;

$Ep\ (\mu l.h^{-1}) = ((E * V * 60))/((T * W))$

Ep: The content of produced ethylene
E: The concentration of ethylene at upper part of glass (µl)
V: The glass volume
T: The time of assay

### 2.5. Total sugar

The total sugar content was measured basis on titration technique and calculated based on following formula:

$S.C\ (\%) = (F \times 0.0095 \times 100)/(V \times 25) \times 100$

S.C: The sugar content (%)
F: The standard sugar factor
V: The volume of extract used for titration

### 2.6. Fruit browning;

The degree of fruit browning was measured by measuring brown pigments in homogenized pulp. The homogenized pulp mixture was kept in glass for 1 hour at room temperature. Afterward, 10 ml of extract and 15 ml of ethanol 95% were mixed. The mixture was centrifuged for 15 min at 800 ×g (BHG optima Π). The absorbance of samples was read at 440 nm based on Coseteng (1987).

### 2.7. Determination of vitamin C content

Determination of vitamin C in apple fruits was done using titration method with 2, 6-Dichloro-Phenol indophenol, and basis on Sharma et al. (2001).

Data analysis was done using SAS V9.2 software. Means comparison was calculated according to the Duncan's multiple range test at 1 and 5 % levels.

## 3. RESULTS AND DISCUSSION

### 3.1. Fruit firmness

Results indicated that fruit firmness was significantly affected by treatments ($P<0.01$). The firmest fruits were produced in $CaCl_2$ (2%) treatment (Fig. 1). The interaction effect of treatments showed that the most firm fruits were produced in 1-MCP ($0.001\ \mu L.L^{-1}$) treatment at 60 days after storage (Fig. 2). The lowest content of fruit firmness was in non-treated apples after 240 days. Different concentrations of 1-MCP did not show a significant effect on fruit firmness during storage (Fig. 2). Fruit firmness is in close relationship with calcium content of fruit and calcium sources offer better tissue integrity than non-treated samples. Calcium nutrition is a very complex phenomenon in plants and fruits have a higher amount of calcium in their tissue comparing other plant organs, therefore, Ca should be absorbed by plant and also transferred to fruits. In general, Ca plays an important role in many physiological aspects, including cell wall stability, cell development, internal processes, membrane stability, and osmotic adjustment. The stability of the cell wall and cell membrane are closely related to the fruit firmness. According to the results of this study, extending storage time decreased fruits firmness, however, 1-MCP and $CaCl_2$ prevented the decrease in fruit firmness and storage life. These results are consistent with Asrey (2012) on mandarin, Hayama (2008) on peach and Mir (2001) on apples, which reported the beneficial effects of 1-MCP in extending storage time. Additionally, our findings are consistent with the results of Akhtar et al. (2012) on Japanese medlar and Gill et al. (2005) on mango, which reported the positive effects of $CaCl_2$ on fruit firmness. The reduction in fruit firmness during storage time, might be related to physiological changes in cell wall, reduction in membrane permeability, and increase in water loss (Roys, 1986). A list of factors including the concentration of $CaCl_2$, treatment duration, fruit maturity, solution temperature, and relative humidity (RH) of storage are important in Ca uptake. Calcium establish an intermolecular crosslink, in the form of pectin polymers, provides resistance to polygalacturonase enzymatic attack. The polygalacturonase enzyme activity is the first reason for conversion of insoluble pectins in to soluble form in middle lamella (Abbot and Conway, 1989). Li et al. (2011) reported that 1-MCP in combination with $CaCl_2$, showed a synergetic effect on decreasing polygalacturonase enzyme activity in jujube fruit (Ziziphus jujuba). Further, application of 1-MCP and $CaCl_2$ on papaya, reduced polygalacturonase and pectin methyl esterase enzymes activity, soluble pectin content, and increased fruit firmness (Nimitkeatkai et al., 2009).

### 3.2. Fruit Browning

Fruit browning was significantly affected by 1-MCP treatment ($P<0.01$). Calcium chloride treatment and storage time did not show significant effect on apple fruit browning. Moreover, the interaction effect of treatments significantly affected fruit browning (Fig. 3). 1-MCP ($0.005\ \mu L.L^{-1}$) and $CaCl_2$ (1%) showed the most positive effects on fruit browning. Fruits with browning indices, show a high activity of polygalacturonase and pectin methyl esterase activity (Manganaris et al., 2007). The susceptibility of fruits to browning depends on ascorbic acid concentration, polyphenol oxidase activity, and phenolic

compounds content. However, some researchers demonstrated that there is a weak relationship between these factors and fruit browning (Nicolas et al., 1994; Weller et al., 1997).

Weller et al. (1997) reported that polyphenol oxidase activity increased during storage and subsequently caused increase in ascorbic acid content. These factors are associated with tissue browning. Probably, the rapid increase in polyphenol oxidase activity related to an increase in respiratory rate due to peeled and cutting fruits (Jeong et al., 2008). Some studies on apples, pears, and pineapple revealed that peroxidase activity may be associated with enzymatic browning (Reddy, 2004). The membrane oxidative damage causes mixing polyphenol oxidase enzyme and the substrate, polyphenols, result in fruit browning which is directly associated with fruit calcium content (Han et al., 2003). Phenylalanine ammonia-lyase enzyme (PAL) is active in the biosynthesis of phenolic compounds pathway, therefore controlling the activity of PAL enzyme, may affect biosynthesis of phenolic compounds and plays an important role in post-harvest control of fruit browning (Martinez and Whitaker, 1995). Ethylene and carbon dioxide stimulate the activity of PAL enzyme and thus increase the production of phenolic compounds. Mahmud et al. (2008) reported that calcium treatment slows down fruit transpiration and therefore reduces ethylene production and thus reduces the activity of the PAL enzyme, subsequently results in reduction the production of phenolic compounds and enzymatic browning of the fruit. These results are consistent with Akhtar et al. (2010) and Hewajulige et al. (2003) on Peach fruits.

**3.3. Total acidity**

Total acidity was significantly affected by treatments as well as interaction effects of treatments ($P<0.01$). Results indicated that increase in storage time caused reduction in total acidity content and fruits after 60 days of storage showed the highest content of total acidity. 1-MCP and $CaCl_2$ treatments significantly increased the content of total acidity. The highest content of total acidity was resulted in 1-MCP (0.001 and 0.005 $\mu L.L^{-1}$) treatments at 60 days of storage and interaction effect of 1-MCP and $CaCl_2$ (2%). The non-treated apples at 180 days after storage showed the lowest content of total acidity (Table 1). The acidity content is related to the concentration of fruit organic acids and is one of the most important factors affecting fruit taste. El-Anany et al. (2009) and Ali et al. (2010) reported that fruit organic acids are the primary substrate for transpiration reactions. The beneficial effect of 1-MCP and $CaCl_2$ on total acidity content might be due to the positive role of this substances in delaying ripening process and also decreasing transpiration (Sigal-Escalada, 2006). Application of $CaCl_2$ reduces ethylene production and transpiration rate, results in decreased metabolism. Our findings are consistent with Akhtar et al. (2010) and Manganaris et al. (2005). Valero et al. (2002) demonstrated that the treatments, which slow down metabolism and delay senescence, result in delaying decrease in total acidity content.

**3.4. Ethylene**

Ethylene amount was significantly affected by treatments. The lowest amount of ethylene was in 1-MCP (0.005 $\mu L.L^{-1}$) and $CaCl_2$ (2%), where control treatment produced the highest amount of ethylene (Fig. 4). In this study, $CaCl_2$ and 1-MCP act as anti-ethylene agents and led to reduced amount of ethylene production. 1-MCP has been introduced as ethylene action inhibitor, which connects to ethylene receptors, with high affinity to this receptors compare to ethylene (10 fold), and inhibits ethylene action (Serek et al., 1995). Moreover, Watkins (2006) reported that 1-MCP extend storage life of apple fruits. Additionally, $CaCl_2$ effectively reduced ethylene production in pineapple (Hewajulige et al., 2003) and mango (Gill et al., 2005).

**3.5. Storage life**

Interaction effect of 1-MCP and $CaCl_2$ significantly affected storage life of apple 'Delbar estival'. The highest storage life was in 1-MCP (0.001 $\mu L.L^{-1}$) and $CaCl_2$ (2%) treatment. Additionally, interaction effect of 1-MCP (0.005 $\mu L.L^{-1}$) and $CaCl_2$ (1%) increased storage life, however increase in $CaCl_2$ amount up to 2% adversely affected storage life (Fig. 5). Results of these study indicated that the effect of $CaCl_2$ treatment on storage life of apple 'Delbar estival' was more effective than 1-MCP. These may be due to the effect of $CaCl_2$ on transpiration reduction, ethylene suppresses, and delaying fruit ripening. Increase in calcium content, changed range of parameters which are effective in senescence such as transpiration, chlorophyll content, and membrane stability (Poovaiah, 1986). Moreover, calcium treatment results in cell wall firmness through increase in number of links between calcium and carboxyl groups of pectin (Hussain et al. 2012). Senevirathna and Daundasekera (2010) reported that application of $CaCl_2$ (2%) increased fruit firmness and shelf life of tomato fruits. Fruits with high SSC showed the higher storage life (Jinquan et al., 2006), which is consistent with our results.

**3.6. Soluble Solids Content (SSC)**

Interaction effect of 1-MCP and $CaCl_2$ significantly affected fruit SSC of apple 'Delbar estival' ($P<0.01$). The soluble solids content was increased as storage life passed and fruits had the highest SSC after 240 days of storage. Application of 1-MCP (0.001 $\mu L.L^{-1}$) increase fruit SSC, however 1-MCP (0.005 $\mu L.L^{-1}$) reduced SSC (Table 2). Application of $CaCl_2$ (1%) resulted in highest fruit SSC. The triple interaction effect of storage time, 1-MCP, and $CaCl_2$ showed that the highest amount of SSC was at 1-MCP (0.005 $\mu L.L^{-1}$) and non-treated apples after 240 days of storage. The same treatment resulted in lowest fruit SSC at 60 days of storage. Increase in SSC might be due to water loss during storage as well as the dissolution of the polysaccharides in the cell wall of mature fruits (Hernandez-Munoz et al. 2006). Increase in soluble solids content with the application of 1-MCP, may be due to break down of complex organic metabolites in to simple molecules or due to hydrolysis of starch to sugars (Will, 1980). Probably, calcium inhibited carbohydrate degradation and thus resulted in higher fruit SSC (Manganaris et al. 2007).

**3.7. Fruit sugar**

Interaction effect of 1-MCP and $CaCl_2$ significantly affected fruit sugar of apple 'Delbar estival' ($P<0.01$). The highest amount of fruit sugar was in interaction effect of control treatment with $CaCl_2$ (1%) at 180 days after storage. Further, the lowest amount of fruit sugar was in interaction effect of control treatment with $CaCl_2$ (1%) at 60 days after storage (Table 3). Results indicated that 1-MCP and calcium treatments did not affect fruit sugar significantly and the effect of storage on sugar content was more important. Overall, ripening results in fruit sugar increase which might be due to increase in hydrolysis enzymes activity and conversion of starch in to sugars. The storage carbohydrate in climacteric fruits is starch which gradually converts in to sucrose after harvest (Hussain et al. 2012). Itai and Tanahashi (2008) showed that application of 1-MCP in Japanese pear (Pyrus pyrifolia Nakai) prevented sucrose reduction and resulted in accumulation of total soluble solids.

**3.8. Vitamin C content**

Vitamin C content was significantly affected by storage time and interaction effect of 1-MCP and $CaCl_2$. Increase in storage time from 60 to 240 days reduced the content of vitamin C about 3% (Fig. 6). 1-MCP significantly increased vitamin C content compare to control. Interaction effect of $CaCl_2$ and control treatment significantly decreased vitamin C content (Fig. 7).

Vitamin C reduction during storage is affecting by range of factors including prolonged storage time, increase in storage temperature, and decrease in relative humidity (Lee et al., 2000). Vitamin C stability is dependent on fruit tissue pH (Veltman et al., 2000). Application of 1-MCP on apple 'Granny smith' reduced oxidative enzymes activity and increased vitamin C content compare to control. Application of 1-MCP prevented reduction of vitamin C in many fruit crops (Watkins, 2006), which is consistent with our results. Spinardi (2005) reported that fruit immersion in calcium, is affective on fruit vitamin C content. Calcium makes crosslinks in membrane and maintain membrane stability through prevent in free oxygen radicals activity. Furthermore, calcium prevents degradation of ascorbic acid (vitamin C) and helps to maintain the antioxidant activity. Vicent et al. (2002) and Mangniriz et al (2007) reported the same results on strawberry and peach, respectively.

## CONCLUSION

1-MCP and $CaCl_2$ showed the positive effects on fruit firmness, soluble solids content, vitamin C content, and storage life of apple 'Delbar estival'. The best effect of treatments resulted in application of 1-MCP (0.001 $\mu L.L^{-1}$) and $CaCl_2$ (2%). Further, 1-MCP (0.005 $\mu L.L^{-1}$) and $CaCl_2$ (2%) extended the storage life of apple fruits efficiently. Results indicated that treatment with 1-MCP and $CaCl_2$ are appropriate treatments for keeping quality of apple 'Delbar estival' in storage.

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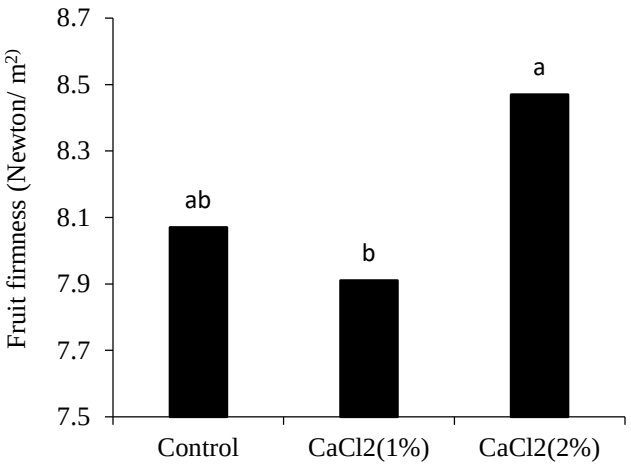


**Figure 1. Effect of calcium chloride on fruit firmness of apple 'Delbar estival'**

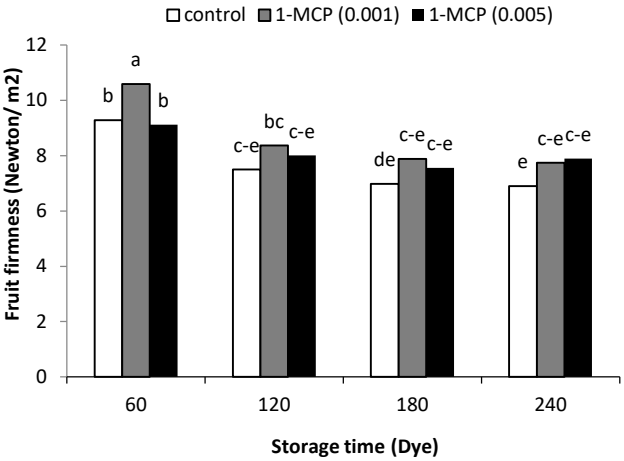


**Figure 2. Interaction effect of 1-MCP and calcium chloride on fruit firmness of apple 'Delbar estival'**

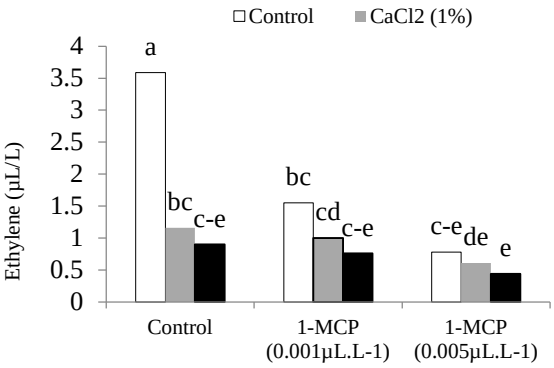


**Figure 4. Interaction effect of 1-MCP and calcium chloride on ethylene production of apple 'Delbar estival'**

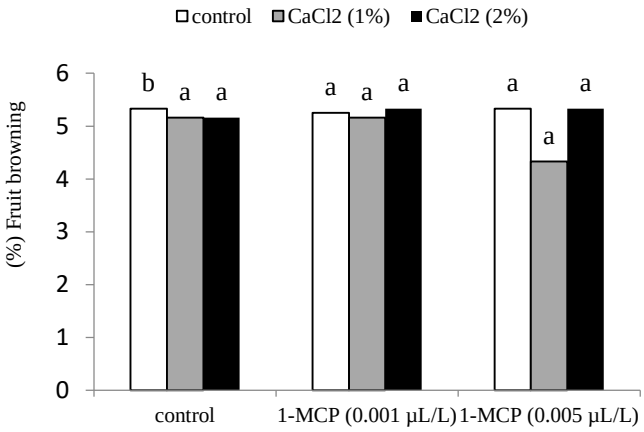


**Figure 3. Interaction effect of 1-MCP and calcium chloride on fruit browning of apple 'Delbar estival'**

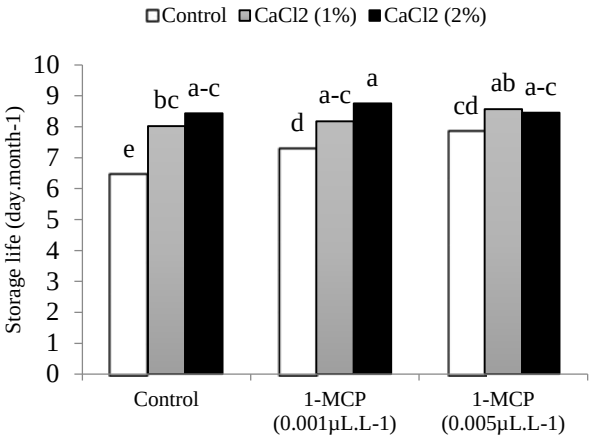


**Figure 5. Interaction effect of 1-MCP and calcium chloride on storage life of apple 'Delbar estival'**

**Table 1. Interaction effects of treatments on fruit total acidity (mg/100g Juice) in apple 'Delbar estival'**

| Storage time | | | | | |
|---|---|---|---|---|---|
| $CaCl_2$ | 1-MCP | 60 | 120 | 180 | 240 |
| | 0 | 1.92 ab | 1.01 l-o | p 0.82 | no 0.97 |
| 0 | 0.001 | 1.81 ab | 1.17 g-k | g-l 1.16 | i-n 0. 9 |
| | 0.005 | 1.69 cd | 1.26 f-g | no 0.97 | j-n 1.08 |
| | 0 | 1.5 e | 1.06 j-n | op 0.88 | op 0.87 |
| 1% | 0.001 | 1.93 ab | 1.14 h-m | m-o 0.99 | g-j 1.19 |
| | 0.005 | 1.6 de | 1.26 f-i | f-i 1.24 | f 1.34 |
| | 0 | 1.7 cd | 0.94 n-p | k-n 1.03 | no 0.98 |
| 2% | 0.001 | 2 a | 1.28 f-g | m-o 0.99 | fg 1.29 |
| | 0.005 | 1.96 a | 1.17 g-k | j-n 1.05 | g-l 1.16 |
| Dissimilar words indicates the significant difference basis on Duncan's multiple range test at 0.05 level | | | | | |

**Table 2. Interaction effects of treatments on SSC (%) in apple 'Delbar estival'**

| Storage time | | | | | |
|---|---|---|---|---|---|
| $CaCl_2$ | 1-MCP | 60 | 120 | 180 | 240 |
| | 0 | 13.87 jk | 14.03 i-k | 14.97 a-e | 14.37 f-k |
| 0 | 0.001 | 14.83 a-f | 14.37 f-k | 14.77 a-g | 14.47 d-i |
| | 0.005 | 14.47 d-i | 14.60 c-h | 14.20 g-k | 14.03 i-k |
| | 0 | 14.63 b-h | 14.47 d-i | 14.80 a-f | 14.87 a-f |
| 1% | 0.001 | 14.70 a-g | 14.80 a-f | 14.53 c-i | 15.10 a-c |

| | | | | | |
|---|---|---|---|---|---|
| | 0.005 | 14.80 a-f | 14.40 e-j | 14.83 a-f | 14.80 a-f |
| | 0 | 13 l | 14.60 c-h | 14.70 a-g | 15.23 a |
| 2% | 0.001 | 13.83 k | 14.97 a-e | 14.47 d-i | 15.03 a-d |
| | 0.005 | 14.50 d-i | 14.90 a-f | 14.10 h-k | 15.20 ab |
| Dissimilar words indicates the significant difference basis on Duncan's multiple range test at 0.05 level | | | | | |

**Table 3. Interaction effects of treatments on Total sugar (mg/100g Juice) in apple 'Delbar estival'**

| Storage time | | | | | |
|---|---|---|---|---|---|
| $CaCl_2$ | 1-MCP | 60 | 120 | 180 | 240 |
| | 0 | 10 gh | 14.68 c-f | 14.35 d-f | 13.59 f |
| 0 | 0.001 | 9.61 h | 15.77 b-e | 15.70 b-e | 15.77 b-e |
| | 0.005 | 11.03 gh | 15.12 c-f | 18.45 a | 14.86 c-f |
| | 0 | 11.19 gh | 17.12 ab | 14.97 c-f | 16.26 b-d |
| 1% | 0.001 | 10.53 gh | 16.19 b-d | 15.18 c-f | 15.51 b-f |
| | 0.005 | 11.65 g | 14.45 d-f | 13.97 ef | 14.45 d-f |
| | 0 | 10.91 gh | 15.44 b-f | 15 c-f | 15.10 c-f |
| 2% | 0.001 | 11.65 g | 14.61 c-f | 14.56 d-f | 14.47 d-f |
| | 0.005 | 10.99 gh | 16.56 bc | 15.26 b-f | 16 b-d |
| Dissimilar words indicates the significant difference basis on Duncan's multiple range test at 0.05 level | | | | | |

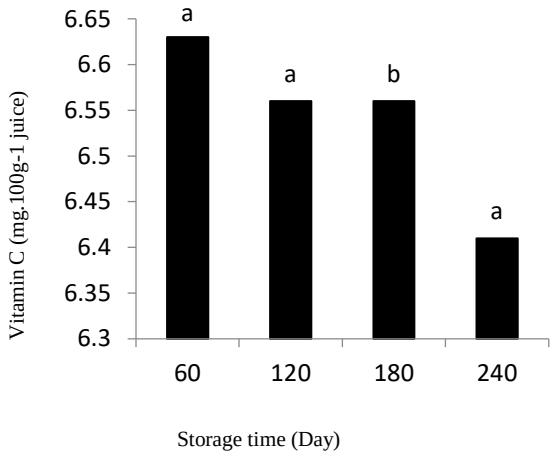


**Figure 6. Effect of storage time on vitamin C content of apple 'Delbar estival'**

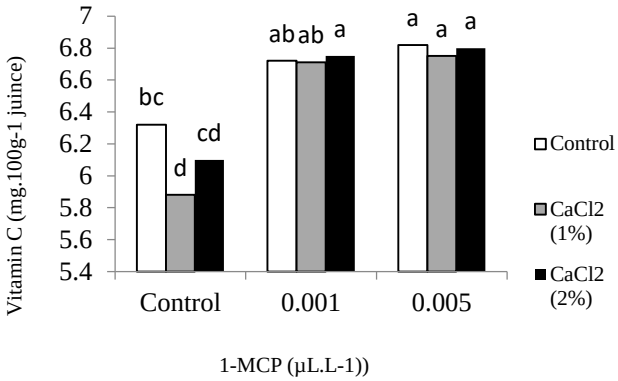


**Figure 7. Interaction effect of 1-MCP and $CaCl_2$ on vitamin C content of apple 'Delbar estival'**