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Research Article

EFFECTS OF PUTRESCENCE, NITRIC OXIDE AND CHLORIDE CALCIUM ON QUALITY ATTRIBUTES OF STRAWBERRY (FRAGARIA ANANASSA DUCH.CV.'CAMMAROSA')

Hoshyar Mohammad Amini and *Nader Habibi
Department of Food Science and Technology, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran
**Author for Correspondence*

ABSTRACT
Because of the harmful effects of the chemicals on human health and environment, the use of these compounds is recently restricted and it is necessary to use the safe compounds in postharvest technology of fruits and vegetables. In this study, the effect of nitric oxide (NO; at concentrations of 3 and 5 µmol L^{-1}), Putrescine (at concentrations of 1 and 2 mmol L^{-1}) chloride Calcium (at concentrations of 25, 50 and 75 mmol L^{-1}) on postharvest life and quality of Cammarosa strawberry fruit during storage at 2.5 □ C with 85-95% RH for 14 days was studied. Total phenolic, total acidity, vitamin C, Fruit softening and Fruit firmness were evaluated. The treatment of fruit with 1, 2 µmol L-1 Putrescine and the treatment with a combination of NO, Putrescine and Chloride Calcium significantly maintained fruit Total Phenolic at the end of the storage. The treatment with a combination of 3µmol L^{-1} NO, 25 and 50 µmol Chloride calcium significantly maintained total acidity at 7 day. The results showed that 3µmol L^{-1} NO and 25 µmol Chloride calcium treatments preserved vitamin C. The treatment with a combination of NO, Putrescine and Chloride calcium significantly prevented fruit softening. In addition, the results indicated that the use of NO, Putrescine and Chloride calcium may be introduced as an effective and successful strategy in postharvest technology of the Cammarosa strawberry fruit. The treatment with Chloride calcium significantly protected firmness fruits.

Keywords: *Strawberry, Putrescine, Nitric Oxide, Chloride Calcium*

INTRODUCTION
Strawberry belongs to the race of Rosacea and the genus of Fragaria and includes 35 varieties. Strawberry, grapes, blueberries and raspberries are types of small fruits among which grapes and strawberry are of more importance and are being cultivated while raspberries and blueberries are wildly grown in different arias (Khoshkhooy *et al.*, 1994). In terms of human nutrition, there are three principal issues as following. First, food must be able to satisfy quantitative needs of our body in terms of calories and satisfaction of hunger. Second, food must be qualitatively balanced; it means that it must supply sufficient amounts of proteins, vitamins, minerals and etc. which lack of any of them causes malnutrition. Third, it should be free from any adverse effects. Vegetables and fruits as a main category of food can help reaching all three aforementioned goals, but it is clear that the importance of a special vegetable or fruit is bound to its special compounds. Researches of the new science of nutrition have proved that consumption of vegetables and fruits prevents several common diseases. As a result, the people of developed countries include more vegetables and fruits in their diets rather than animal products (Asniashari and Zakaei, 2009a).
Quality conservation and increasing the durability of vegetables and fruits is extremely important and is mainly related to the status of sales markets. On this basis, in order to increase profitability, conservation of the quality of products is necessary. Also satisfaction of consumer needs for several types of vegetables and fruits during the year is only possible through long term storage of products. A large portion of agriculture products are wasted before they reach consumer markets (Asniashari and Zakaei, 2009a).
Harvested products are usually highly putrescent and without application of suitable technics and methods, their capability for transportation and maintenance for consumption in all seasons, adjustment of markets and shipment to distant markets declines. In addition, nowadays there is a high emphasis on the nutritional value of vegetables and fruits and in order to attract global consumers, there must be vast

researches undertaken regarding increasing the nutritional value of vegetables and fruits. Meanwhile, health of food is the first important index in evaluation of food in a way that not only the products should be free from diseases and pests, but also existence of any kind of chemical residues in them is inacceptable and application of chemical compounds which are harmful for the environment and humane health is seriously questioned (Asqari, 2007; Rahemi, 1995).

With respect to high putrescent attributes of strawberry, on one hand it is necessary to implement sound methods for decreasing wastes and increasing the shelf life of the product and on the other hand, since the interval between harvesting and consuming this product is short, application of chemicals in post harvesting technology of strawberry is impossible because the applied chemicals will not have enough time to decompose and respectively will enter the consumer's body. On this basis, finding healthy and non-chemical materials is the first research priority in strawberry's post harvesting physiology (Asniashari and Zakaei, 2009a).

The purpose of this research is to investigate the effects of post harvesting application of Nitric Acid, Chloride Calcium and Putrescine (as a polyamine) in conservation of qualitative attributes of Cammarosa strawberry and preservative role of these compounds in preventing decomposition of fruit's advantageous compounds such as Vitamin C and phenolic compounds.

MATERIALS AND METHODS

Methods

Chemical Compounds

Gallic acid, Folin-Ciocalteau, Sodium Carbonate, Ascorbic Acid, 2, 6-Dichlorophenol indophenol, Sodium Nitroprussiate Dihydrate, Metaphosphoric Acid, 1, 4-D-Amino Butane and Hydroxide Sodium purchased from Sigma Company located in St. Louise, MO, USA.

Treatment with Putrescine

Fruits were treated with Putrescine via a immersing method for two minutes and were placed in plastic dishes after drying and were transferred to a refrigerator at 2 degrees and relative humidity of 85-95 percent.

For this research, strawberry fruits were immersed in a solution containing 0, 1 and 2 MM of 1, 4-D-Amino Butane.

Treatment with Chloride Calcium

For this purpose, Cammarosa strawberries were immersed in 0, 25, 50 and 75MM solutions of Chloride calcium for two minutes and afterwards, were dried in lab atmosphere and were kept in a refrigerator at 2 degrees for 14 days.

Treatment with Nitric Acid

For this purpose the Cammarosa strawberries were sprayed with 0, 3 and 5MM solutions of Sodium Nitro-Pruccide which releases Nitric Acid. After the treatment, fruits were put in a refrigerator on a plastic dish at 2 degrees for 14 days with humidity of 85 to 95 percent.

For undertaking each of the aforementioned treatments, a number of 15 healthy strawberries without any physical deformities and abnormal shape were selected.

Ultimately, the control fruits were also treated with distilled water.

Fruit's Total Phenol

Measurement of Phenolic compounds was completed via Folin-Ciocalteau as following (Waterhouse, 2002). First the strawberry juice was diluted with distilled water with the ratio of 10 percent. 1mm of strawberry juice, different standard values of Gallic Acid and 1mm of Blanc (Deionized or distilled water) were mixed in a container and respectively 70mm water was added to the solution. Afterwards 5mm of Folin-Ciocalteau reactor was mixed with the solution and next, the solution was kept in room temperature for 3 to 5 minutes. After that, 15mm of Sodium Carbonate solution was added to the mixture and the total weight of the solution was reached to 100mm by adding extra water. Ultimately 4mm of the yielded solution was putted in Spectro-photometer with a wavelength of 765nm and the absorption was recorded.

Titratable Acidity

For determining the amount of Titratable acids, the Titration method with 0.1 normal benefits was used. For this purpose, 10mm of filtered solution was putted in a 250ml flask and 40ml of distilled water and a few drops of Phenolphthalein were added to the solution and next, it was normalized with 0.1 benefits. When the color of the solution changed to pink and was stabilized for about 30 seconds, the amount of consumed benefits was determined (Nadeem *et al.*, 2010).

Firmness of Fruit Texture

For determining the firmness of fruit's texture the TA-XTplus device (made by England's Stable Micro System Company) was used. For this purpose, the single axle penetration test was applied. The pre-test speed of the device was set to 2mm per second and it test speed was set to 1mm per second and also the post-test speed of the device was set to 10mm per second. The used Probe was a cylindrical stainless steel type with flat base and diameter of 6 millimeters. The probe's displacement was set to 10 millimeters. The values of force with an accuracy of 0.1g, distance with an accuracy of 0.001mm and time with an accuracy of 0.001 second were recorded and the maximum required force for penetration was readout from time-force vectors (Vargas *et al.*, 2006).

Vitamin C

For determining the value of vitamin C, the Titration method and the solution of 2, 6-Dichlorophenol indophenols were used and finally the value of vitamin C was calculated on the basis of milligrams of Ascorbic Acid in 100 g sample (Husseini, 1991).

Statistical Analysis

The test was undertaken in a factorial design on the basis of a pure random method with 11 treatments and 3 repetitions in two periods of 7 and 14 days. Statistical analyses were undertaken via the software of SAS 9.1 and comparisons of mean were carried out through Duncan's multiple range test.

RESULTS AND DISCUSSION

Results and Conclusion

Since the sampling and evaluation of attributes were carried out in two different times, in addition to applied material, the element of time is also introduced to calculations. Two sampling times are as follows:

Time1: seventh day after storage in refrigerator

Time2: fourteenth day after storage in refrigerator

Table 4-1: Results of analysis of the variance of the effects of treatments, storage time and mutual effects on measured attributes and Cammarosa strawberry

Square means					**Degrees of (df)freedom**	**Changes sources**
deterioration	**firmness**	**Vitamin C**	**acidity**	**Total phenol**		**S.O.V**
122/581**	893/727**	21/556**	0/0037**	44039/79**	10	Treatment (main factor)
1/343	11/483	0/2067	0/000057	31/75	40	Treatment's test error
11307/92**	74942/06 **	5201/19**	0/04313**	1090065/51**	1	Storage time (subsidiary factor)
89/19**	689/59**	13/69**	0/0017**	29351/64**	10	(A×B)Treatment x time
0/209^{ns}	2/ 56^{ns}	0/371 ^{ns}	0/00025^{ns}	1/ 879^{ns}	2	(R×B)Repetition x time

Fruit's Total Phenol

The content of phenolic compounds was declined in all treatments at the end of storage time and this declination was lower in treated fruits with first compositional treatment and Putrescine in both densities. Among the implemented materials, the first compositional treatment (putrescence 1MM + Chloride Calcium 50 MM + Nitric Oxide 3MM) and Putrescine treatments were more effective in maintenance of

total Phenol in both densities. This is while the 3MM and 5MM Nitric Oxide had the least effectiveness in maintenance of total Phenol in treated fruits.

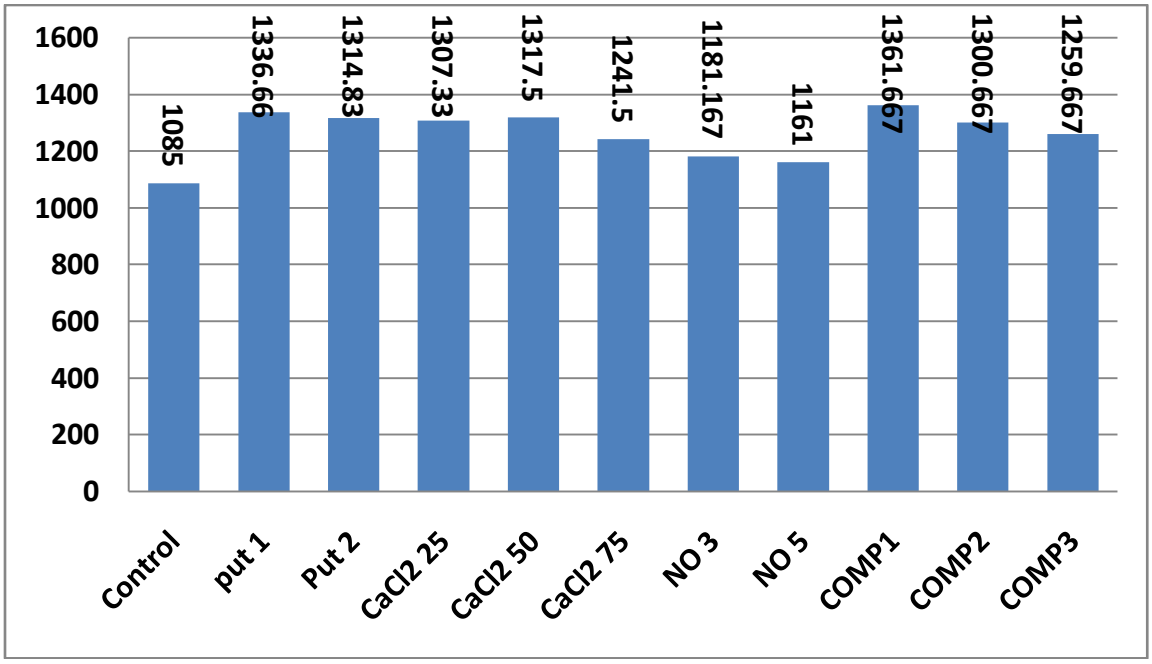

Figure 1: The effect of chemical treatments on total phenol of Cammarosa strawberry

Put1: 1MM Putrescine, put2: 2MM Putrescine, CaCl2 25: 25MM Chloride Calcium, CaCl2 50: 50MM Chloride Calcium, CaCl2 75: 75MM Chloride Calcium, NO3: 3MM Nitric Oxide, NO5: 5MM Nitric Oxide, COMP1: 1MM Putrescine + 50MM Chloride Calcium + 3MM Nitric Oxide, COMP2: 2MM Putrescine + 75MM Chloride Calcium + 5MM Nitric Oxide, COMP3: 1MM Putrescine + 25MM Chloride Calcium + 3MM Nitric Oxide, Control: Control treatment

In the present research, the content of Phenolic compositions had declined for all treatments at the end of storage time and this declination was lower in treated fruits and therefore, the entire undertaken treatments had a significant effect on inhibiting the reduction of total Phenol value except for distilled water treatment.

It seems that treatment with Putrescine and Chloride Calcium inhibits the deterioration of total Phenols via inhibiting the activity of Enzymes which dissect Phenolic compositions such as Poly Phenol Oxidase and respective reduction in production of ethylene.

It is also been said that concentrations of Nitric Acid of less than 1MM per liter impose a preventive effect of the activity of Poly Phenol Oxidase and Phenyl alanine Ammonialiase (Zoo *et al.*, 2009b).

Treatment with Chloride Calcium and Putrescine decreases aspiration and as a result declines the production and effects of Ethylene and also leads to a decreased activity of Phenyl alanine Ammonialiase and ultimately prevents loss of total Phenol value (Hivido, 1978).

Valero *et al.*, (1997) indicated that treatment of pomegranate with Polyamines leads to preservation of the density of Ascorbic Acid and increased Phenolic compositions compared to control fruits. This suggests the decrease of activities of Phenol Oxidase Enzyme and as a result, the brownish effect decreases. Their findings are in compliance with the results of Doan *et al.*, (2007).

Effects of Chemical Treatments on Acidity

According to variance analysis table 1-4 it could be inferred that implemented chemical treatments in Cammarosa strawberry fruits are significant at 0.01 in terms of acidity. Control group fruits featured the lowest level of acidity while among the implemented treatments; maximum acidity value was related to

the treatment of 25MM Chloride Calcium and 3MM Nitric Oxide which were both ranged in one statistical group with no difference.

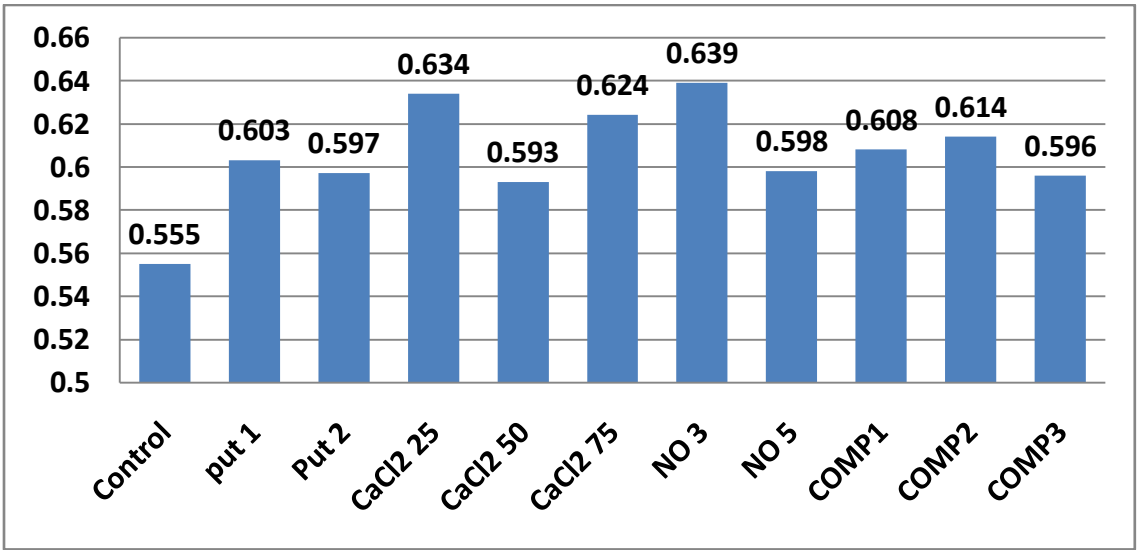

Figure 2: Effects of chemical treatments on the acidity of Cammarosa strawberries

Put1: 1MM Putrescine, put2: 2MM Putrescine, CaCl2 25: 25MM Chloride Calcium, CaCl2 50: 50MM Chloride Calcium, CaCl2 75: 75MM Chloride Calcium, NO3: 3MM Nitric Oxide, NO5: 5MM Nitric Oxide,COMP1: 1MM Putrescine + 50MM Chloride Calcium + 3MM Nitric Oxide, COMP2: 2MM Putrescine + 75MM Chloride Calcium + 5MM Nitric Oxide, COMP3: 1MM Putrescine + 25MM Chloride Calcium + 3MM Nitric Oxide, Control: Control treatment

Application of Putrescine also leads to a lower declination of acids compared to control treatment which points to the role of Putrescine in maintenance of acids in the fruits of strawberry and pomegranate (Serrano *et al.*, 2003). During the post-harvest period, the values of acidity and PH are respectively expected to decrease and increase.

Research conducted by Chewer *et al.*, (1991) revealed that strawberries which were treated with Calcium had lost less acidity after 14 days of storage. This could be as a result of lowered speed of fruit's ripening process because of application of Calcium which leads to decomposition of organic acids to other compositions.

Results of this research are not in compliance with the results of researches conducted by Zakaei *et al.*, (2009). They stated that Putrescine has no significant effects on inhibition of loss of acidity in strawberries and apricot.

Effects of Chemical Treatments on the Level of Vitamin C

According to variance analysis table 1-4, chemical treatments have a significant effect on the level of Vitamin C at significance level of 0.01; in a way that according to means comparison table, the compositional treatment of 5MM Nitric Oxide had the most effective influences on maintenance of the level of Vitamin C. on the other hand, the existing data in aforementioned table shows that control group fruits and fruits treated with 1MM Putrescine had the lowest level of Vitamin C. results of this test indicated that all there compositions were significant at 0.01 in maintaining the level of acidity. This difference was bolder in seventh day. Among the treatments, the 3MM Nitric Oxide in seventh day and applied compositional treatments in 14^{th} day had the most effects in maintaining the acidity.

As a result of consumption during aspiration, organic acids decrease while the fruit ripens and their decrease is directly related to metabolic activities. In fact, organic acids are reserved sources of energy for the fruit which are consumed at the time of ripening with increase in metabolism (Rahemi, 2006). Studies indicate that elements that cause decreased aspiration and production of ethylene prevent loss of organic

acids as well as increase of solid particles in solutions as a result of reduced sugar consumption (Rahemi, 2006). In this research, application of Nitric Oxide significantly affected the maintenance of level of acidity. Nitric Oxide leads to decrease of cell's metabolic activities such as aspiration and production of ethylene and therefore, the loss of acidity decreases.

According to the results of Sing *et al.*, (2008) treatment with Nitric Oxide has significantly postponed the loss of total acidity which complies with our results in this research.

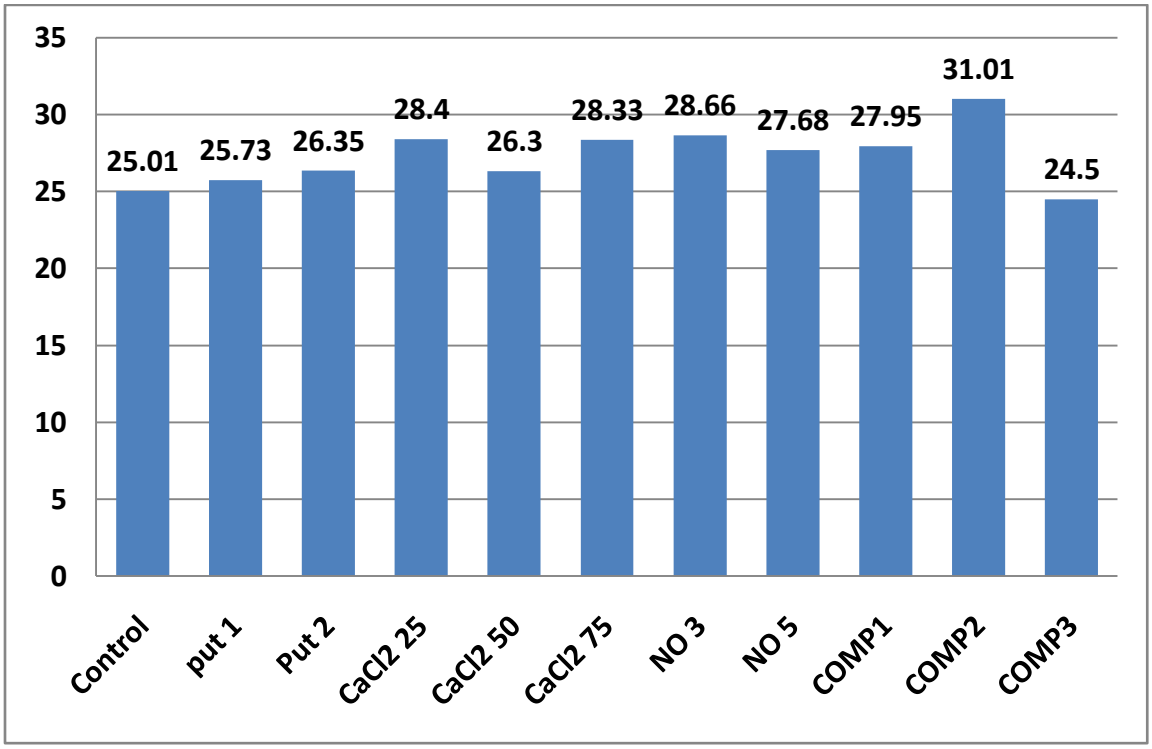

Figure 3: Effects of chemical treatments on Vitamin C levels of Cammarosa strawberry

Put1: 1MM Putrescine, put2: 2MM Putrescine, CaCl2 25: 25MM Chloride Calcium, CaCl2 50: 50MM Chloride Calcium, CaCl2 75: 75MM Chloride Calcium, NO3: 3MM Nitric Oxide, NO5: 5MM Nitric Oxide,COMP1: 1MM Putrescine + 50MM Chloride Calcium + 3MM Nitric Oxide, COMP2: 2MM Putrescine + 75MM Chloride Calcium + 5MM Nitric Oxide, COMP3: 1MM Putrescine + 25MM Chloride Calcium + 3MM Nitric Oxide, Control: Control treatment

Presence of relatively high values of vitamin C in strawberry has turned this fruit into one the most desirable products in the market. On this basis technics and solutions for maintaining the values of vitamin c as well as prolonging its post-harvest life can play a significant role in post-harvesting process of strawberry.

During the storage period the value of Ascorbic acid which is a main anti-oxidant decreases. The reason is consumption of this vitamin as a provider of electron for oxidants for diffusing free radicals (Semimov, 1995). Decreased vitamin C content can be a result of activity of enzymes such as Ascorbic Acid Oxidase.

These enzymes are more active in cut tissues (Clean, 1987).

Putrescine leads to a linear decrease in the level of Ascorbic Acid which is under the influence of Polyamine treatments through increased activity of Ascorbic Oxidase. Decrease in total Oxidant levels is a result of increased activities of Cytochrome Oxidase, Ascorbic Acid Oxidase and Peroxidase Enzymes. Reduction in color development in external application of Putrescine is related to a reduced deterioration of Chlorophyll and postponed aging process. Polyamine treatments are effective on the ratio of weight

loss which significantly increased the ratio of solid materials in the solution compared to acids during the process of ripening. Treatment of pomegranate fruits with polyamines led to maintenance of density of Ascorbic Acid and increased Phenolic compounds compared to control group fruits as a sign of reduced activity of Phenol Oxidase Enzymes and respectively decreased browning effect (Mirdehqan *et al.*, 2007). Chloride Calcium and Nitric Oxide also prevent decomposition of cell walls and restrict production of free radicals through preventing the production of ethylene, reduction of aspiration and delaying aging and therefore, as a result of reduced free radicals the cell's need for consumption of Ascorbic Acid also decreases and overall, the levels of vitamin C are maintained in the fruit (Semimov, 1995). It's been reported that the vitamin C content of Lebanese yellow apples treated with Chloride Calcium are significantly higher than control group fruits. This fact could be attributed to a postponed fast Oxidation of vitamin C by Chloride Calcium (Akhtar *et al.*, 2010).

Results of this research are in compliance with the results of researches conducted by Abdulahi *et al.*, (2011) regarding strawberry.

Effects of Chemical Treatments on Firmness

According to variance analysis table 1-4, the performed treatments significantly affected fruit's firmness at the significance level of 0.01. According to means comparison table 3-4 it can be observed that among the performed treatments, the control group fruits had the least firmness while the firmness of other performed treatments was significantly highest than the control group. Results of this test indicated that among the compositional treatments, treatments which were compositions of three different materials showed the highest level of firmness after 7 and 14 days of storage. On the day 14, treatment of 75MM Chloride Calcium had also a positive effect on maintaining the firmness of fruits along other compositional treatments.

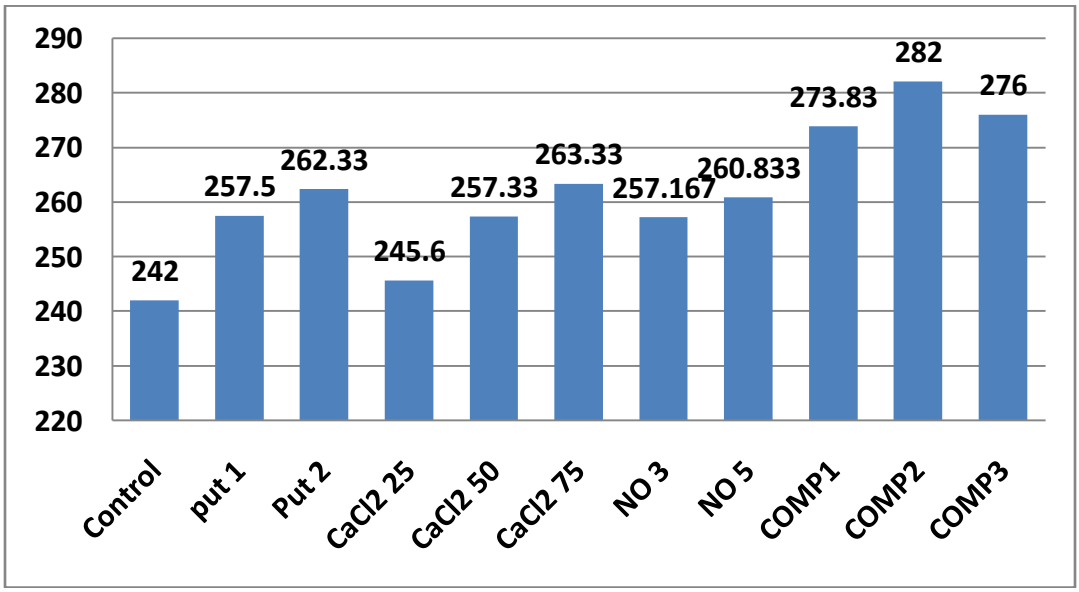

Figure 4: Effects of chemical treatments on firmness of Cammarosa strawberry

Put1: 1MM Putrescine, put2: 2MM Putrescine, CaCl2 25: 25MM Chloride Calcium, CaCl2 50: 50MM Chloride Calcium, CaCl2 75: 75MM Chloride Calcium, NO3: 3MM Nitric Oxide, NO5: 5MM Nitric Oxide,COMP1: 1MM Putrescine + 50MM Chloride Calcium + 3MM Nitric Oxide, COMP2: 2MM Putrescine + 75MM Chloride Calcium + 5MM Nitric Oxide, COMP3: 1MM Putrescine + 25MM Chloride Calcium + 3MM Nitric Oxide, Control: Control treatment

During the storage period, enzymes such as Pectinestrase, Cellulase and etc. lead to decomposition of cell walls and a respective decrease in product's firmness (Hernandez *et al.*, 2008). Aging, decomposition of cell walls and loss of weight are among factors which lead to decreased firmness of products and loss of

marketability (Vargas *et al.,* 2006). Results of this research indicated the positive effects of Calcium on optimization of firmness of the tissue of samples. The main role of Calcium in strengthening membranes of plant tissues reflects in different ways. A large portion of Calcium embeds in the walls of plant tissues. This unique condition of calcium is a result of several places of consolidation of calcium in the cell walls and its extremely restricted displacement from the membrane of cytoplasm to the inside of the cell's cytoplasm. On the middle edge, calcium is connected to carboxyl groups related to Pectin and the solution's Pectate calcium decreases. On the other hand the existing Pectate on the cell walls of organic plants is decomposed by Poly Galactronase enzyme. High concentrations of calcium severely reduce the activity of the enzymes in charge of this composition. Therefore, with increase of the value of calcium in the tissue, the activity of this enzyme reduces and the decomposition of cell walls slows down. Therefore, with increase in the level Calcium present in the tissue, the activity level of this enzyme reduces and decomposition of the cell wall slows down. Calcium also causes cellular stability by attaching to groups of Phosphates, Carboxylates, Phospholipids and proteins on the surface of the membranes (Chewer, 1991). Calcium is known as an intermolecular attaching element in stabilization of the middle edge's pectin protein complex; in addition calcium reduces the softness of the tissue via preventing the process of solubility.

By being established inside the cell wall as an intermolecular connector which stabilizes the compounds of the middle edge, calcium preserves the structure of cell wall. On the other hand, calcium influences the structure and task of the cellular membrane and by connecting the enzyme proteins and non-enzyme proteins to the phospholipids of the cell membrane plays its role. In this way, reduces the activity of ethylene producing enzymes which also have a protein like structure. Ultimately, with a reduced level of ethylene production, which is the stimulator of the activity of enzymes which hydrolyze the cell wall the cell wall suffers a reduced amount of damage and calcium treated fruits will remain firm. On this basis, by establishment inside the cell wall and stabilizing it and also reducing the amount of ethylene production, calcium plays its role in maintaining the firmness of fruit's membrane (Wang *et al.,* 1993).

The destruction of pectates is carried out by poly-Galactronase enzymes and when there are sufficient amounts of calcium, their destruction is prevented (Reddy *et al.,* 2004) and in this way, plant shows resistance against destruction of cell walls. The operation mechanism in this case is that although the most of the calcium is established inside the cell wall, the production of ethylene is related to destruction of the plant due to destruction of the cell wall but treatment with calcium strengthens the cell wall and reduces aspiration leads to reduction and minimization of the level of produced ethylene (Convey *et al.,* 1984). In addition, treatment with calcium chloride reduces the damages due to low temperature and freezing through preserving the fluidity of membrane and fixing the ratio of polyunsaturated fatty acids to saturated fatty acids and ultimately leads to firmness of plant's membranes (Quills *et al.,* 2004).

The effect of Putrescine on increasing the firmness of fruit's pomace could be related to its bonding with pectin compounds of the cell wall. This bonding leads to stability and fixation of cell's wall which is detectible right after treatment. The aforementioned bonding also prevents the activity of enzymes which decompose the cell wall including PME, PE and PG (Valero *et al.,* 2002). Decrease in softness of fruit by Putrescine could be could be as a result of reduction of activity of enzymes which decompose the cell wall including Ando Poly Galactronase, Exo poly Galactronase and Methyl-Esterase. Increase in the firmness of fruit in treatment by Polyamines: Polyamines raise on negative loads of phospholipid compounds or anionic areas on the membranes and therefore, multiples the stability of these membranes. The bonding of polyamines with pectin materials restricts the accession of enzymes which decompose cell walls to pectin materials. Application of 1MM Putrescine on Plum fruits during the storage period in at 10 degrees of temperature led to maintenance of membrane's firmness and the result was increased storability of the fruit. Immersion of fruits in Putrescine solution led to removal of Fungi's spore and reduces contaminations and the surface of polyamines effectively increases in leaves contaminated with black spots, fungal diseases and gray mold which show the role of these compounds in plant's pervasive reactions against pathogens. It's been reported that pre harvest treatment with Putrescine on peaches increased the firmness of the fruit and delayed their ripening (Bergoli *et al.,* 2002). Results of this

research comply with the findings of researches conducted by Punapa *et al.*, (1993) on strawberries and Serrano *et al.*, (2003) on plum fruits.

Effects of Chemical Treatments on the Level of Wilting

According to variance analysis table 1-4, the applied chemical treatments have significantly affected the prevention of wilting at the significance level of 0.01. Results of this research indicated that the wilting level of treated fruits with all three compounds was significantly less than control fruits. According to yielded information, there were no significant differences between compounds on the seventh day of testing. This was while, on the fourteenth day, the treatments of calcium in all three concentrations showed the least wilting level.

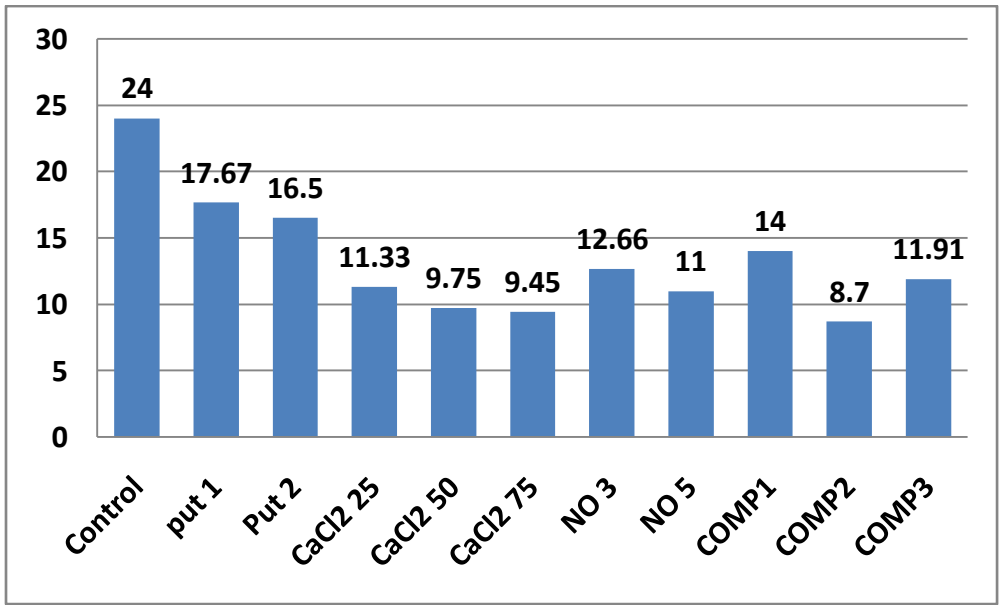

Figure 5: Effects of chemical compounds on wilting level of Cammarosa strawberry

Put1: 1MM Putrescine, put2: 2MM Putrescine, CaCl2 25: 25MM Chloride Calcium, CaCl2 50: 50MM Chloride Calcium, CaCl2 75: 75MM Chloride Calcium, NO3: 3MM Nitric Oxide, NO5: 5MM Nitric Oxide,COMP1: 1MM Putrescine + 50MM Chloride Calcium + 3MM Nitric Oxide, COMP2: 2MM Putrescine + 75MM Chloride Calcium + 5MM Nitric Oxide, COMP3: 1MM Putrescine + 25MM Chloride Calcium + 3MM Nitric Oxide, Control: Control treatment

The yielded results indicated that as the level of absorption of calcium in the fruit is increased, the level of wilting is decreased. The Poly-Galactase enzyme leads to decomposition of all pectin compounds inside the fruit except for the cell wall and as a result provides a site for fungal contamination therefore, treatment with chloride calcium in addition to strengthening the membranes of the fruit, also prevents fungal deterioration.

Through firming the cell wall, calcium leads to increased strength against enzymes produced by fungi. Also, calcium increases the level of Oxalate and soluble pectin (Gupta *et al.*, 1980).

During the past 20 to 25 years, researchers have found out that increase in fruit's calcium level in addition to increasing the firmness of the membrane, causes a decrease in the level of wilting. In a test, Delicious apples were treated by 8% calcium solution via stressed penetration method and were kept in storage for three months. Afterwards, fruits were inseminated with blue mold after storage period and the signs of wilting in calcium treated plants were recorded as being approximately 40% less than control group fruits. After 5 months of storage there was no difference between treated fruits and control group fruits (Convey, 1983).

Results of research conducted by Marconi *et al.*, (2002) on strawberries and cherries comply with the findings of this research. Investigating the level of contamination of tomato showed that the low

concentration of calcium in raw extracts was accompanied with higher level of contamination. Also in storage, the suitability of resistance against physiological and fungal diseases is related to fruit's deterioration (Malakooti, 2000). On the other hand, it's been reported that plant's sensitivity against contamination by pathogenic elements is in a converse relation with the calcium levels of plant's membranes (Malakooti, 2000; Hagh, 1993). Chang *et al.,* (1993) sprayed the solution of calcium chloride in concentrations of 0.3 and 0.9 percent two weeks before harvesting and observed the significant effects of these treatments on reducing the level of fruit's deterioration. On the other hand, the durability of Scoya strawberries was increased from 3 days to 16 days without any type of contamination (Desuza *et al.,* 1999). According to the theory of Convey and Sam (1987) the present calcium in the cell wall highly protects the plant from microbes which try to enter the fruit via breaking the pectin. Some performed studies on different varieties such as apples, pears and pineapples revealed that also Peroxidases might be related to enzyme induced browning. Although, the quality of these effects is not clear but this issue, as it was declared by Pouya (1988) and Pikchioni *et al.,* (1995), could be a result of the fact that calcium helps the stability of the membranes. Nitric Oxide stimulates the defensive responses of host membrane and might also have a direct impact on growth of pathogens or even indirectly increase the resistance of host against the pathogen (Quadir and Hashinga, 2001). Different evidence has indicated that Nitric Oxide synthesizes as a result of pathogens invasion by Nitric oxide and could corporate with H2O2 for the purpose of establishing defensive reactions (Neil *et al.,* 2003). It's also been reported that Nitric Oxide plays a role in development of systematic acquired resistance (Dornier *et al.,* 1998) and probably there is a signaling relation among H2O2, NO and Salicylic Acid during ultra-sensitive reactions and systematic acquired resistance (Romero *et al.,* 2003). Leshm and Pinchasso (2000) conducted a research and showed that application of Nitric Oxide leads to an increases storability and decreased deterioration. Production of Nitric Oxide has a converse relation with production of Ethylene in fruit's maturity and ripening stages. Leshm *et al.,* (2000) reported that strawberry's storability was increased by Nitric Oxide vapor. Yang and Hoffmann (1984) reported that surface of ACC are the restrictor factors in ethylene's production cycle. Results of this research are in compliance with the findings of other researchers in this context.

Table 2: effects of chemical treatments on qualitative attributes of Cammarosa strawberry

Properties					
Deterioration (Percentage)	Firmness (G-Force)	Vitamin C Mm Ascorbic Acid In 100g Sample	Acidity G Per 100ml Citric Acid	Total Phenol Mg Acid) Gallic Per (Liter)	**Treatment**
17.68^{B}	257.50^{D}	25.01^{G}	0.603^{DE}	1336.66^{B}	**1MM Putrescine**
16.50^{B}	262.33^{C}	25.73^{F}	0.597^{E}	1314.83^{C}	**21MM Putrescine**
11.33^{DE}	245.6^{E}	26.35^{E}	0.6345^{A}	1307.33^{D}	**25MM Chloride Calcium**
9.75^{FG}	257.33^{D}	28.40^{BC}	0.593^{E}	1317.5^{C}	**50MM Chloride Calcium**
9.45^{G}	263.33^{C}	26.30^{E}	0.624^{B}	1241.5^{G}	**75MM Chloride Calcium**
12.66^{D}	257.167^{D}	28.33^{BC}	0.639^{A}	1181.167^{H}	**3MM Nitric Oxide**
11.00^{EF}	260.83^{CD}	28.66^{B}	0.598^{E}	1161.0^{I}	**5MM Nitric Oxide**
14.00^{C}	273.83^{B}	27.68^{D}	0.608^{DC}	1361.66^{A}	**1MM Putrescine + 50MM Chloride Calcium**
8.7^{G}	282.00^{A}	27.95^{CD}	0.614^{C}	1300.667^{E}	**2MM Putrescine + 75MM Chloride Calcium + 5MM Nitric Oxide**
11.91^{ED}	276.66^{B}	31.01^{A}	0.596^{E}	1259.66^{F}	**1MM Putrescine + 25MM Chloride Calcium + 3MM Nitric Oxide**
24^{A}	242.00^{E}	24.50^{G}	0.555^{F}	1085^{J}	**Control**

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