FOLIAR SPRAY OF NUTRIENTS AFFECTS FRUIT QUALITY, POLYGALACTURONIC ACID (PECTIN) CONTENT AND STORAGE LIFE OF PEACH FRUITS IN TURKEY

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Abstract. Fruit flesh softening is generally accompanied by changes in pectin structure in most fruits. In cell wall, pectin polysaccharides cross-linked with Ca^{2+}. Especially Calcium chloride application is environmentally friendly and is also very effective on fruit quality. An experiment was performed by foliar spraying of calcium, magnesium and manganese alone and in combination to assess their effects on fruit quality, storage life and the polygalacturonic acid (pectin) content in peach (*Prunus persica* cv. JH. Hale). Experiment was conducted in commercial peach orchard located in Çanakkale, Turkey. Treatments included 1.5% $CaCl_2$, 2% $MgSO_4$, 1% $MnSO_4$, combination (0.6% $CaCl_2$, 1% $MgSO_4$ + 0.1% $MnSO_4$), and control (water) applications. Spray applications were started 30 days after the full bloom, and continued at four-week intervals up to four weeks before the harvest. The amount of pectin in the peach fruits was determined by FT-IR spectroscopy. Fruit weight loss, firmness, color, titratable acidity, pH and soluble solids contents were determined. Foliar nutrient-treated trees showed improved resistance to compression and penetration, as well as a decrease in weight-loss during postharvest storage. A similar response was obtained from Mg treatments. The smallest weight loss occurred in calcium and manganese applications, which were 0.71 and 0.75%, respectively. Calcium and magnesium treatments had a positive effect on fruit flesh firmness. The highest polygalacturonic acid content was obtained with $CaCl_2$ treatment, which was 414% greater than that of control treatment. Therefore, $CaCl_2$ application can be safely used to increase pectin content as well as fruit quality of peach fruit.
Keywords: *pectin, polygalacturonic acid, FT-IR spectra, peach, calcium chloride, storage*

Introduction

Short shelf life in peach fruit after harvest is due to rapid ripening which results in a limitation for efficiency in handling and transportation. Climacteric fruit species practically undergoes softening after textural changes during postharvest storage (Harker et al., 1997). Physicochemical and biochemical changes affect fruit's final flesh firmness (Brummell et al., 2004). Consumer acceptability is mostly influenced by fruit texture, which is largely established by cell wall and middle lamella polysaccharides (Roeck et al., 2008).

Treatments such as wrapping, cold storage and high amount of CO_2, calcium compounds, aminoethoxyvinylglicyne and 1-MCP have been used to improve fruit quality (Sisler and Serek, 1997; Byers, 1997; Fan et al., 2002; Manganaris et al., 2007). Pre and postharvest treatments with calcium chloride has been proven to be safe and environmentally friendly (Karabulut et al., 2003). Additionally, application of foliar sprays is environmentally friendly fertilization method since the nutrients directly contact to the plant without little contamination to soil (Farrag et al., 2015). Integrated fruit production systems frequently utilize preharvest calcium sprays to improve fruit characteristics and to minimize fungicide applications towards the end of harvest by improving resistance to brown rot (Conway et al., 1994). Calcium deficiency is associated with many physiological disorders in fruits. Although calcium level can be

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increased with foliar sprays, in many cases, achieving it proves to be difficult due to its limited uptake and absorption by the fruit and transport into the fruit (Mengel, 2002).

In plant cells, pectin, a structural polysaccharide, is mostly present as protopectin. The backbone of pectin consists of, in part, 1-4 linked galacturonic acid residues. Quantitative and structural analyses of complex polysaccharides are required for accurate evaluation of the content of galacturonic acid (Gary, 2004). Cell wall strength is affected by calcium in the cell wall because it involves in producing cross-bridges and is considered to be the last barrier before cell degradation (Fry, 2004). Plant cell wall is stabilized by exogenous Ca applications and protected from cell wall degrading enzymes (White and Broadley, 2003). Marscher (1995) stated that stability of cell wall is increased with Ca ions by linking non-esterified pectins and that fruit senescence might be due to its applications. Beavides (2011) reported delayed softening in apple and pear fruits during storage period, following Ca applications before harvest.

Mg^{2+}, a bivalent cation like Ca^{2+}, might sustain cell wall and plasma membrane integrity through acting as a bonding agent between pectin substances in the cell wall (Farag and Nagy, 2012). Lester and Grusak (1999) reported that Ca and Mg treatments influenced weight loss and fruit firmness during storage. Weight loss was linked with fruit deterioration during postharvest handling (Gonzales-Aguilar, 2009). Crisosto et al. (1999) stipulated that Ca sprays may have result in skin discoloration in peach and nectarine.

Literature about the effects of manganese on fruit species are limited to their applications as foliar and/or soil supplement to determine effects on fruit quality in species such as peach and nectarine (Serrano et al., 2004) and orange (Labanauskas et al., 1963). However, these studies did not include its possible effects on fruit quality during storage.

Mansoor et al. (2001) reported that FTIR can be used as an alternative method for determining pectin content in commercial pectin samples and in pectin extract. Since FTIR requires no reagent, rapid, cost and time effective application of FTIR method for fruits could be useful.

The objective of this study was to rapid determination of different foliar nutrient applications ($CaCl_2$, $MgSO_4$, and $MnSO_4$) on pectin content and fruit quality of peach (*Prunus persica* L.) by using FT-IR spectroscopy.

Materials and Methods

Plant material and location

Field experiments were conducted on a commercial orchard located in Lapseki-Çanakkale, Turkey (40° 19.8′ N, 26° 43.8′ E, around 47 m above sea level). The orchard soil is sandy loam and was classified as Typic Xerofluvents according to Soil taxonomy.

The average annual rainfall is about 616 mm and the mean temperature is about 15°C (Anonymous, 2017). Ten-year-old peach trees (*Prunus persica* cv. J.H. Hale) growing under a drip irrigation system, were selected for study. Trees were managed according to commercial practices for fertilization, cultivation, irrigation, pest and weed control, and hand thinning of fruits was also performed.

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Treatments

Eight suitably distant sub-plots (5 trees each) were selected for applications. Trees received each of the following different leaf treatments: 1.5% $CaCl_2$ (75 g/5 L), 2% $MgSO_4$ (100 g/5 L), 1% $MnSO_4$ (5 g/5 L), combination (0.6% $CaCl_2$, 1% $MgSO_4$ + 0.1% $MnSO_4$), and control (water (5 L) was sprayed) (Alcaraz-Lopez et al., 2003 ; Farag and Nagy, 2012).Four leaf applications at 4 week intervals were performed as follows: 1^{st} application on April 26^{th}, 30 day after the full blooming; 2^{nd} application on May 22^{nd}; 3^{rd} application on June 20^{th}, and 4^{th} application on July 18^{th}, 2012. Applications were ended 4 weeks before the harvest. Fruits were harvested at firm-ripe stage depending on the skin ground color as maturity index. After the elimination of defective fruits, all treatments were placed in 5 kg commercial plastic containers and then placed in a storage room at 0°C and 90% relative humidity for 30 days. The fruits were analyzed at 10 day intervals during cold storage. Three replicates were utilized for each trial, with 30 fruits per replicate.

Weight loss (%) was determined using a 0.001 g precision balance (Precisa XB 220A) at 10 days intervals. Weight loss was calculated as: (Wi-Wf) / Wi x 100, where Wi was the initial sample weight and Wf was the final sample weight. The results were expressed as percentage weight loss. Peach tissue firmness was measured with a penetrometer (Chatillon, model DFS-500, USA), after removing skin, using a flat-head stainless-steel cylindrical probe with a 8 mm diameter. Penetration depth of the probe into the flesh of peach fruit was approximately 3 mm (the flesh thickness of the samples ranged between 3.5 and 5.5 mm for cv. J. H. Hale). Maximum force (N) and MT slope (N mm^{-1}, ratio of force to the deformation until the maximum force) extracted from the force/deformation were considered to be measures of fruit firmness.

The color (hue angle) was measured using a Chroma meter (Minolta CR-400, Japan). The color of each fruit was measured in terms of the L*, a* and b* coordinates, and from these values, the hue angle was calculated as $h^{\circ} = \arctan(b/a)$ (Abbott, 1999).

For the purpose of determination of titratable acidity (TA), pH and soluble solids content (SSC), samples were taken from each treatment and pulped by using a blender. SSC (°Brix) was determined by a digital refractometer (Kyoto Electronics Manufacturing Co. Ltd., Japan, and Model RA-250HE) at 22°C. TA was determined by means of titration with 0.1 N NaOH until pH reached 8.1, expressing the results in of g malic acid/100 ml. The pH was determined by potentiometric measurement at 22ºC with a pH meter (WTW, Germany). All measurements were done at the beginning of the trial and every other 10 days for a 30 day storage period.

Determination of galacturonic acid content

The infrared absorption spectra were obtained from a Perkin Elmer BX II spectrometer in KBr discs and were reported in cm^{-1} units. Spectra were collected by co-adding 32 scans at a resolution of 0.5 cm^{-1} in 4000-400 cm^{-1} range. Standards for polygalacturonic acid were purchased as KBr from Sigma-Aldrich (Steinheim, Germany). Polygalacturonic acid (PGA) calibration standards were prepared by mixing polygalacturonic acid with potassium bromide to cover a range of acid concentrations (10-98%). High-purity water from a Millipore Simplicity 185 water purification system (Millipore Iberian S.A., Madrid, Spain) was used for all chemical analyses and glassware washing. A set of 10 calibration polygalacturonic acid standards was prepared by blending polygalacturonic acid with KBr to obtain acid standards with polygalacturonic acid content of 10, 20, 30,

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40, 50, 60, 70, 80, and 98%, respectively. Pectin (PGA) was extracted by the method of Kratchanova et al. (2004) from peach samples.

The experiment was factorial and completely randomized design with 3 replicates for each treatment. The data was evaluated by using SAS® (1990) statistical package program and groupings were done using Duncan’s multiple range test, at 5% importance level.

Results

Table 1 shows the weight loss during a 30 day-cold storage period at 0°C. The smallest weight loss occurred in calcium and manganese applications (0.71 and 0.75%, respectively), whereas highest weight losses were obtained from the magnesium, combination, and control fruits (1.00, 0.91and 0.89%) respectively.

Table 1. *Effects of preharvest foliar applications on weight loss (WL%), fruit flesh firmness (N) of peach (cv. J. H. Hale) fruits during the cold storage conditions (0^0C, 30 days).*

Parameters	Treatment/days	0	10	20	30
WL (%)	Control	0^{NS}	1.25a*	0.98ab	1.34ab
	Calcium	0	0.76bc	0.85b	1.22b
	Magnesium	0	1.28a	1.15a	1.55a
	Manganese	0	0.69c	0.86b	1.42ab
	Combination	0	0.96b	1.17a	1.51a
	Mean	0	0.99	1.00	1.41
Flesh Firmness (N)	Control	55.00c	34.43b	20.00b	6.64^{NS}
	Calcium	65.00a	40.38a	32.00a	7.53
	Magnesium	64.67a	31.75b	25.04b	7.23
	Manganese	61.67ab	29.76b	22.03b	6.39
	Combination	58.33bc	29.33b	21.03b	6.24
	Mean	60.93A	33.13B	24.02C	6.81D

*Means within columns with the same small letter are not significantly different at the $p < 0.05$ level. NS: Non-significant.

Significant reduction in fruit flesh firmness was detected during the cold storage period for all treatments (*Table 1*). However calcium and magnesium treated fruits had less reduction in fruit flesh firmness compared to other treatments. At harvest, applications of calcium (65 N) and magnesium (64.16 N) greatly improved fruit flesh firmness. On the other hand, the rest of the treatments, including the control, provided the lowest firmness in the fruits. The reduction was significantly greater in the combination, manganese and control fruits compared to those in the calcium and magnesium treatments.

An interaction effect was found on L* color parameter (*Table 2*). This effect was observed only in the Ca treated fruits at the 30th day of the storage period. L value reached 68 at the end of the storage period (*Table 2*). Ca positively affected the brightness of the fruits and enabled them to be brightest at the end of the storage. Hue*, on the other hand, was influenced by the treatments but prolonged time of storage caused a significant decrease at the end of the storage. Hue* values were between 112.50 and 115.30 but the differences were not statistically significant (*Table 2*).

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Table 2. *Effects of preharvest foliar applications on L* and Hue* angle of peach (cv. J. H. Hale) fruits during the cold storage conditions (0^oC, 30 days).*

Parameters	Treatment/days	0	10	20	30
L*	Control	63.17a*	62.02a	61.58a	63.60b
	Calcium	62.06b	66.50a	79.92a	68.47a
	Magnesium	64.16a	62.90a	63.06a	63.23b
	Manganese	65.72a	63.26a	60.85a	66.27b
	Combination	63.33a	62.10a	60.81a	62.60b
	Mean	63.69	63.36	65.24	63.83
Hue*	Control	116.36^{NS}	116.56^{NS}	115.81^{NS}	114.77^{NS}
	Calcium	114.90	115.01	115.04	113.90
	Magnesium	116.09	115.87	115.46	115.31
	Manganese	116.15	114.99	118.92	115.15
	Combination	115.21	115.29	115.43	112.50
	Mean	115.74A	115.54A	116.1A	113.58B

*Means within columns with the same small letter are not significantly different at the $p < 0.05$ level. NS: Non-significant.

SSC and pH of the fruits were dependent on both applications and storage period (*Table 3*). SSC showed an increase and decrease trend during storage period. It seems that nutrient treatments caused a delay in accumulation of sugar components until 20th day of the storage, after which all treatments had similar ratios of SSC. Differences among the treatments showed that no specific treatment sustained its SSC throughout the storing period. Interestingly, the gradual increase was more prominent in the $CaCl_2$ and combination treated fruits compared to the control ones. Calcium treated fruits contained the highest pH (4.11) throughout the storing time, followed by the control fruits (3.90) up to the 20th day but later it drastically stayed the lowest (3.92) compared to the rest of the applications. Titratable acidity in the fruits was affected by the treatments. The highest titratable acidity was obtained from the magnesium (0.825%) followed by calcium (0.758%), control (0.728%), combination (0.698%) and manganese (0.655%) treatments (*Table 3*).

Table 3. *Effects of preharvest foliar applications on SSC(%), pH and TA of peach (cv. J. H. Hale) fruits during the cold storage conditions (0^oC, 30 days).*

Parameters	Treatment	0	10	20	30
SSC (%)	Control	13.13bc*	13.75a	13.00b	13.00cd
	Calcium	13.25bc	13.50b	13.60a	13.75a
	Magnesium	13.50a	13.58b	13.50a	13.50b
	Manganese	13.50a	12.75c	12.75c	12.90d
	Combination	13.00d	12.25d	13.00b	13.10c
	Mean	13.28	13.17	13.17	13.25
pH	Control	3.78b	3.89a	3.90b	3.92d
	Calcium	3.87a	3.87a	4.00a	4.11a
	Magnesium	3.56d	3.78c	3.80d	3.98c
	Manganese	3.74c	3.82b	3.85c	3.95cd
	Combination	3.74c	3.79bc	3.84c	4.05b
	Mean	3.74	3.83	3.88	4.00

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TA (g L^{-1} malic acid)	Control	0.805^{NS}	0.750^{NS}	0.751^{NS}	0.607^{NS}
	Calcium	0.645	0.850	0.790	0.748
	Magnesium	0.860	0.830	0.813	0.798
	Manganese	0.720	0.650	0.630	0.620
	Combination	0.720	0.701	0.690	0.682
	Mean	0.750A	0.756A	0.735A	0.691B

*Means within columns with the same small letter are not significantly different at the $p < 0.05$ level. NS: Non significant.

FT-IR spectra and total carbonyl absorption peak area at 1740-1635 cm^{-1}, from free COO- and esterified COO-R groups of polygalacturonic acid samples, were obtained using the method described for polygalacturonic acid standards (*Table 4*). A linear relationship between polygalacturonic acid content and carbonyl absorption band area was found ($R^2=0.982$).

Table 4. *The carbonyl absorption area of the polygalacturonic acid (PGE) standards*

Polygalacturonic acid (%)	**FTIR carbonyl peak area**
10	22,87
20	29,97
30	40,43
40	47,52
50	55,57
60	63,17
70	69,59
80	78,41
90	81,66
98	83,23

Polygalacturonic acid contents of peach fruits were calculated from the linear fit equation (*Table 5*, *Fig. 1*). The calculated polygalacturonic acid (PGA) content of 17 peach samples were; 87.67, 83.79, 78.23, 74.42, 70.45, 65.94, 58.78, 53.58, 52.56, 46.67, 45.23, 43.36, 21.13, 20.18, 19.14, 15.78, 15.75 %, respectively . Polygalacturonic acid content was affected by $CaCl_2$, $MgSO_4$, $MnSO_4$ and $CaCl_2$-$MgSO_4$-$MnSO_4$ mixture applications and subsequently increased an average of 414% with $CaCl_2$, 340% with $MgSO_4$, 302% with $MnSO_4$ and 262% with $CaCl_2$-$MgSO_4$-$MnSO_4$ compared with the control (*Fig. 2*, *Table 5*).

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Table 5. *The calculated polygalacturonic acid (PGE) content of the peach samples.*

Peach samples[a]	Polygalacturonic acid (%)
1	87,67
2	83,79
3	78,23
4	74,42
5	70,45
6	65,94
7	58,78
8	53,58
9	52,56
10	46,67
11	45,23
12	43,36
13	21,13
14	20,18
15	19,14
16	15,78
17	15,75

[a] Peach trees were sprayed with containing 1.5% $CaCl_2$, 2% $MgSO_4$, 1% $MnSO_4$ and 0.6% $CaCl_2$-1% $MgSO_4$-0.1% $MnSO_4$. Day 0: 1 (Ca^{2+}), 2 (Mg^{2+}), 3 (Mn^{2+}), 4 (Combination), Day 10: 5 (Ca^{2+}), 6 (Mg^{2+}), 7 (Mn^{2+}), 8 (Combination), Day 20: 9 (Ca^{2+}), 10 (Mg^{2+}), 11 (Mn^{2+}), 12 (Combination), Day 30: 13 (Ca^{2+}), 14 (Mg^{2+}), 15 (Mn^{2+}), 16 (Combination), 17 (Control, Chemical-free).

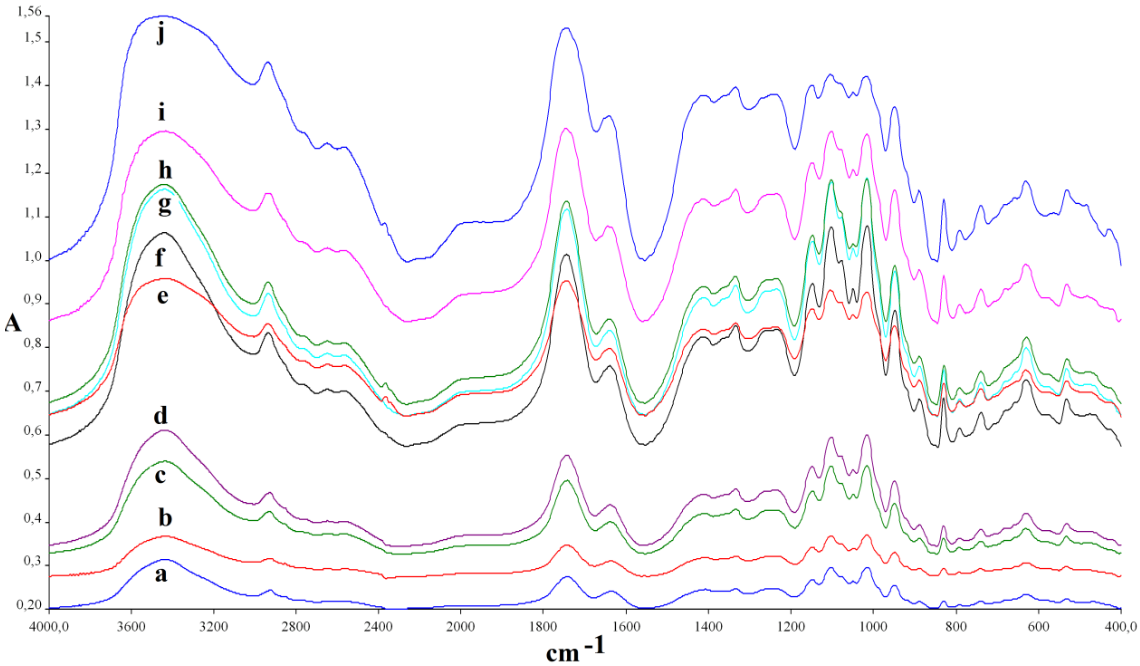

Figure 1. *FTIR spectra of the 4000-400 cm^{-1} region of polygalacturonic acid standards diluted with KBr : (a) 10, (b) 20, (c) 30, (d) 40, (e) 50, (f) 60, (g) 70, (h) 80, (i) 90, (j) 98%.*

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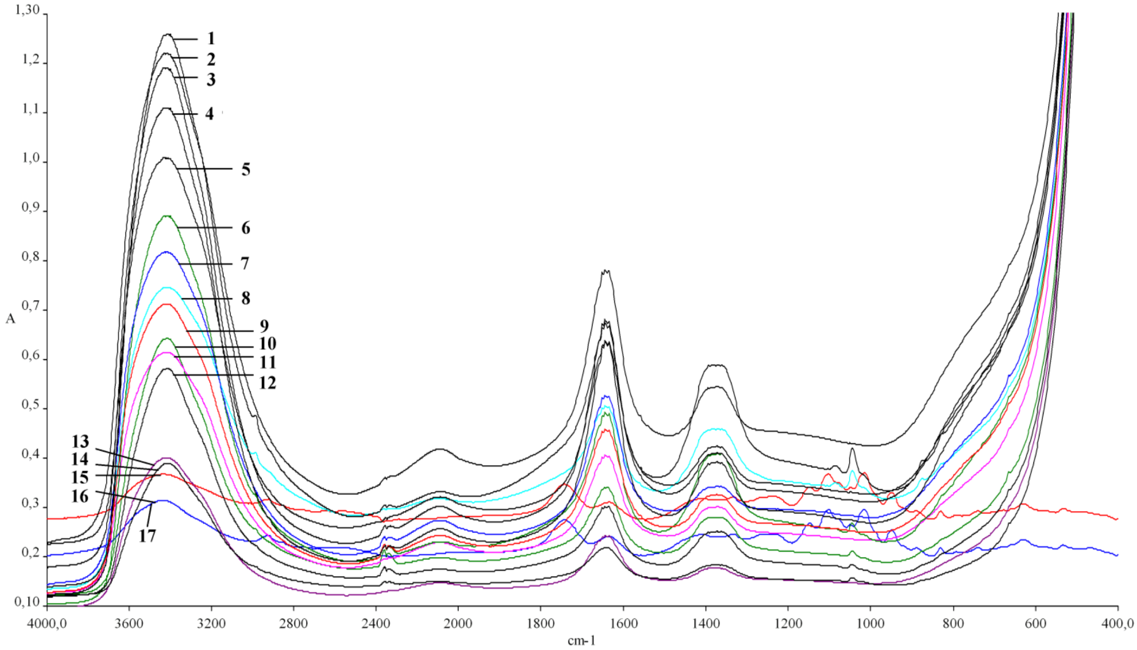

Figure 2. *FTIR spectra of the peach samples sprayed with 1.5% $CaCl_2$, 2% $MgSO_4$, 1% $MnSO_4$ and 0.6% $CaCl_2$-1% $MgSO_4$-0.1% $MnSO_4$. Day 0: 1 (Ca^{2+}), 2 (Mg^{2+}), 3 (Mn^{2+}), 4 (Combination), Day 10: 5 (Ca^{2+}), 6 (Mg^{2+}), 7 (Mn^{2+}), 8 (Combination), Day 20: 9 (Ca^{2+}), 10 (Mg^{2+}), 11 (Mn^{2+}), 12 (Combination), Day 30: 13 (Ca^{2+}), 14 (Mg^{2+}), 15 (Mn^{2+}), 16 (Combination), 17 (Control, Chemical-free).*

Discussion

Effects of the treatments on the commercial quality of cv. J. H. Hale fruits are discussed with specific focus on improving their mechanical properties. Parameters related to fruit quality were evaluated starting from harvest at ten day intervals up to 30th day under storage. Weight loss is a consequence of fruit dehydration and leads to loss of quality and associated fruit deterioration during postharvest handling (Gonzales-Aguilar et al., 2009). In this research, calcium and manganese treatments reduced weight loss compared to other treatments. Treatments might have delayed fruit senescence, therefore limiting the water loss. Calcium was reported to retard ripening (Liu et al., 2009) and protect cell membrane integrity (Guimarães et al., 2011).

The increased water loss in Mg, combination and control fruits might have affected cell turgor and be related to the lower firmness detected. According to Marschner (1995), calcium ions increase the stability of cell walls by binding non-sterified pectins even though plant cell walls are permeable to water. In melons, fruit firmness was affected by Ca and Ca-Mg applications (Lester and Grusak, 1999). Preharvest calcium treatment of trees was found to be useful in delaying pear and apple softening during the storage (Benavides et al., 2001).

Ca applications increased the L value of peaches . Crisosto et al. (1999) stated that Ca spray formulations may contribute to peach and nectarine skin discoloration depending on application rates. Ground color of the skin in these genotypes is closely associated with the ripeness of the fruit in general and the flesh firmness in particular (Kader, 1999). Kao et al. (2012) reported a decrease in H* value with progressing of

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flesh softening throughout the ripening. In our research, the lowest Hue* value was obtained from the combination treatments, however, they were not statistically different.

$CaCl_2$ and combination treatments increased SSC contents during the storage in this study. Similarly, Manganaris et al. (2007) reported that effects of both 62.5 mM and 187.5 mM $CaCl_2$ applications increased SSC at the end of the storage period. Values for the pH also reacted the same, being lower at the harvest and gradually increasing towards the end of the storage.

Foliar applications of mineral nutrient elements had significant effects on the biochemical and physical properties of the fruits. FTIR spectroscopy analysis showed that the structure of pectin extracted from fruit is similar to the commercial pectin spectrum. For the analysis of pectin, the absorbance peaks at 1000- 1600 cm^{-1} was focused. Polygalacturonic acid content was affected by $CaCl_2$, $MgSO_4$, $MnSO_4$ and combination applications and subsequently increased an average of 414-262% compared with the control. It also decreased during the cold storage**.** Ekinci and Yildiz (2015) determined PGA contents of sweet cherry fruits using FTIR and showed that pre-harvest foliar Ca applications after bloom resulted in highest PGA content at harvest. Monsoor et al. (2001) reported the usefulness of FTIR method to determine pectin content of commercial samples.

The reduced weight losses in the treated fruits could be due to maintaining the cell membrane integrity. Lester and Grusak (1999) observed positive effects of Ca and Ca-Mg treatments for weight loss in melons during storage.

TA losses during the peach storage show that fruit quality is decreasing. TA losses in Ca and Mg applications are less than in other applications. However, these values were not statistically significant. Manganaris et al. (2007) reported that all storage time reduced TA of peach fruits in all treatments.

In this research it was shown that polygalacturonic acid content changed during the storage. At the harvest, the fruits contained the highest content during which the storage time it gradually diminished. In consistent with our results, Mignani (1995) showed that polygalacturonase in tomato pericarp was reduced by calcium.

Conclusion

Preharvest foliar nutrient applications were important for peach fruit quality. Especially 1.5% $CaCl_2$ sprays provided an increase in pectin (PGA) content of fruits at harvest. Calcium applications increased tissue firmness and pH at harvest. It maintained tissue firmness, pH, and TA during the cold storage. Additionally, Ca application decreased weight loss of fruit. Fruit color was preserved during the storage. The smallest weight loss occurred in 1.5% $CaCl_2$ and 1% $MnSO_4$ applications.

$MgSO_4$ (2%) application helped to keep fruit flesh firmness and TA value but it was not as effective as Ca application. Combination treatments had greater concentration than other treatments and this caused a deformation of fruit epidermis, leading to an increase in both weight loss and fruit color. Lower concentrations of $CaCl_2$ needs to be studied for future studies. A linear relationship between polygalacturonic acid content and carbonyl absorption band area was found. Using FTIR method to determine PGA contents of fruits is suggested since FTIR requires no reagent, and is rapid, cost and time effective compared to standard chemical methods.

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