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Evaluation the Preharvest Application of Iron and Nitrogen on Some Qualitative Characteristics of Two Apple Cultivars during Cold Storage

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Abstract

Apple is one of the most important fruits in temperate zones with a long post-harvest life during cold storage. A factorial experiment, in a randomized completely block design was used to investigate the preharvest application of Iron (0, 500 and 1000 mg Fe-EDDHA per tree) and nitrogen (0, 48 and 96 g ammonium nitrate per tree) on some postharvest qualitative characteristics in "Red spur" and "Granny smith" apple cultivars. Iron and nitrogen treatments in all 72 trees were applied in three stages (full bloom, 30 and 60 days after full bloom). Fruit quality parameters including colorskin color parameters (L*, a*, b*), flesh firmness, total soluble solids (TSS ,(Titratable acidity (TA) ,flavor index) TSS/TA (and total antioxidant activity were measured .According to the results, a decrease in fruit firmness, TA and increase in TSS/TA in both cultivars were observed during cold storage irrespective of treatments. The results showed that in "Red spur" cultivar, Fe application decreased fruit firmness, TSS, b* parameter values and increased TSS/TA, a* parameter values, while, N application increased TSS. Fruit firmness was decreased and TSS, TSS/TA, b* parameter values were increased by Fe application in "Granny smith" cultivar. Fruit firmness, TSS and L* parameter values were decreased and TA was increased by N application on this cultivar. In conclusion, Fe and N application in proper levels improved some postharvest qualitative characteristics in "Red spur" and "Granny smith" apple cultivars during cold storage.

Keywords: Cold storage, Fruit quality, Iron, Nitrogen, Red spur and Granny smith.

Abbreviations: cv, Cultivar; N, Nitrogen; TA, Titratable acidity; TSS, Total soluble solids.

Introduction

Apple (*Malus domestica* Borkh.) is one of the most important fruit trees of the world that belongs to the Rosaceae family (Ganai et al., 2015). This fruit have been one of the human diets since ancient times (Ashoori et al., 2013) and represent a good source of dietary fiber, pectin, potassium,

and vitamins A and C (Savatović et al., 2008). Apple is a climacteric fruit with a long post-harvest life in cold storages (Rabiei et al., 2012). New postharvest technologies are developed to extend the storage life of fruits (Roth et al., 2003).

Fruit quality can be affected by several preharvest orchard cultural practices (Fallahi et al., 2001). Mineral nutrition is one of the preharvest factors that influence

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apple quality before and after storage (Johnston et al., 2002). Quality aspects and physiological disorders of fruit are also related to the mineral composition of the apple fruit (Tahir et al., 2007). Nitrogen management is critical for achieving high yield and good fruit quality in commercial apple production, because tree growth, fruit yield and quality are dependent on N (Wang and Cheng, supply 2011). nitrogen Although, high fertilization increases yield, it is of great important to estimate optimum nitrogen requirement of the tree to avoid negative effects on fruit quality during storage and to prevent environmental strains such as groundwater pollution (Milic et al., 2012).

On the other hand, the most prevalent nutritional disorder in fruit trees that are grown in calcareous soils is chlorosis induced by Fe deficiency (El-Jendoubi et al., 2011). High HCO₃ concentration and pH in calcareous soil are two main factors responsible for low Fe availability in soil and Fe efficiency in plants (Huang et al., 2012). Iron deficiency is a common nutritional disorder in many crop plants, resulting in poor yields and reduced nutritional quality (Rout and Sahoo, 2015). This deficiency is particularly important in fruit tree species, causing decreases in vegetative growth of tree, fruit yield and quality losses and a decrease in the life span of orchards (Abadía et al., 2011). Iron chlorosis has deleterious effects on fruit production in some fruit tree. This disorder is affecting fruit quality parameters such as colorcolor, firmness, or acidity (Álvarez-Fernández et al., 2009). There is scientific evidence that Fefertilization increases fruit quality and vield in many crops (Álvarez-Fernández et al., 2006). Therefore, Fe fertilizers, either applied to the soil or delivered to the foliage, are provided to crops every year to control Fe deficiency (Abadía et al., 2011).

However, there are few studies about influence of preharvest application of N and Fe on fruit quality during storage. For that reason, the main objectives of the

present work were to evaluate the effect of different levels of nitrogen and Iron nutrition on some postharvest qualitative characteristics in two apple cultivars.

Materials and methods

Plant material and experimental design

The study was carried out in an orchard located in the Horticultural Research Station of the University of Tehran, Karaj, Iran during 2015. The average altitude, annual temperature, sunshine days and rainfall are 1321 m, 14.4 °C, 203 day and 247.3 mm respectively. The soil had a loamy texture with 11.46 % total CaCO₃, 1.03% organic matter, 0.1 % total nitrogen and pH of 7.75.

Twenty two years old apple trees, cultivars "Red spur" and "Granny smith" which grafted on M9 and M26 respectively were selected for the experiment. Trees received 250 g chemical fertilizers (ammonium sulfate, triple superphosphate, urea and sulfur per tree) mixed with animal manure yearly in April and drip irrigation is done after full bloom twice a week.

A factorial experiment, in a randomized completely block design with replications was used to investigate the effects of three levels of nitrogen (0, 48 and 96 g ammonium nitrate per tree) and three levels of Iron (0, 500 and 1000 mg Fe-EDDHA per tree) on some postharvest qualitative characteristics of fruit. The fertilizer, ammonium nitrate (NH₄NO₃) and Fe-EDDHA (Sequestrene 138-Fe) were applied to soil in a stripe of 50 cm at each side of the row. Iron and Nitrogen treatments in all 72 trees were used in three stages (full bloom, 30 and 60 days after full bloom) during 2015.

At the commercial harvest time for each cultivar (first and end of September for cultivars "Red spur" and "Granny smith", respectively), 20 fruits of each tree were randomly selected, cleaned and put in a plastic bag and then transformed to cold storage of Department of Horticulture Sciences, Faculty of Agriculture and

Natural resources, University of Tehran. Fruits were stored in a cold storage (4±0.5 °C and 85-90% relative humidity (RH)). After 45 and 90 days ("Red spur" cultivar) and 30, 60 and 90 days ("Granny smith" cultivar) storage, fruits were taken from cold storage for fruit quality assessments.

Physicochemical analysis

Color and firmness were assessed individually in five fruits per tree. Flesh firmness was measured on both sides of fruit using a penetrometer (FT 327) with a 11 mm diameter probe.

Fruit skin color was determined with a colorimeter (Model Konica Minolta CR-403, Japan). Color changes were quantified in the L*, a*, b* color space. L*, refers to lightness of the color and ranges from black = 0 to white = 100. A negative value of a* indicates a green color where the positive value indicates red-purple color. A positive value of b* indicates a yellow color and the negative value a blue color.

Titrable acidity (TA) (as malic acid) was determined by titration of 10 ml juice by 0.1N NaOH up to pH of 8.1. Total soluble solid contents were measured by extracting and mixing one drop of juice from five fruit into a refractometer (RF40) and expressed as 'Brix. Fruit flavor index (TSS/TA) was determined by ratio of TSS to TA.

Antioxidant activity

Total antioxidant activity was measured by using the method of Faniadis et al. (2010) as this method one g of fruit tissue homogenized with 80% methanol. The mixture was centrifuged at 12000 rpm for 15 min.

Reaction mixtures containing 3400 μL of DPPH (1-diphenyl-2-picrylhydrazyl) and 100 μL methanol extract held at room temperature in darkness for two h. The absorbance of the reaction mixtures was read at 520 nm on the spectrophotometer. The capability of total antioxidant activity was calculated using the following equation:

Antioxidant activity $\% = (A_{520} \text{ blank} - A_{520} \text{ sample } /A_{520} \text{ blank}) * 100$

Statistical analysis

The statistical procedure was performed by SAS software (Version 9.1(and mean comparisons was performed using Duncan's test at $P \le 0.05$. Graphs were plotted using Microsoft Excel 2010.

Results

Fruit Firmness

According to Figure 1, fruit firmness of "Red spur" cultivar was significantly decreased with increasing storage time so that it was the highest in fresh harvested fruits while the least in fruits stored for 90 days. A similar trend was found in "Granny smith" cultivar from second month (Fig. 1). The fruit firmness in "Red spur" cultivar was decreased by Iron treatment at 1000 mg. N application also significantly changed fruit firmness. The highest and lowest values were found in 96 and 48 g/tree respectively (Table 1). In "Granny smith" cultivar both Iron levels decreased fruit firmness and the lowest value (74.8 N) was found in 1000 mg Iron treatment. Fruit firmness was decreased by N application at 96 g/tree (Table 3).

Table 1. Effects of Iron and nitrogen on fruit firmness, TSS, TA and TSS/TA in "Red spur" cultivar

Treatme	nts	Fruit Firmness (N)	TSS (°Brix)	TA (%)	TSS/TA
Inon	0	66.9 a	13.96 a	0.308 b	46.22 b
Iron	500	66.3 a	14.05 a	0.302 a	45.94 b
(mg/tree)	1000	63.9 b	13.51 b	0.280 c	49.3 a
NT	0	65.5 b	13.73 b	0.294 a	47.44 a
Nitrogen	48	64.6 c	14.12 a	0.303 a	47.36 a
(g/tree)	96	67 a	13.67 b	0.293 a	46.66 a

Within each column, means followed by the same letters are not significantly different by the duncan test at $P \le 0.05$.

Treatments		L*	a*	b*	Total antioxidants (%)
T	0	64.53 a	2.97 b	34.02 a	35.47 a
Iron	500	62.78 a	7.58 a	32.71 b	33.7 a
(mg/tree)	1000	63.47 a	3.08 b	34.29 a	37.44 a
Nitus	0	63.87 a	3.77 a	33.93 a	37.48 a
Nitrogen	48	62.85 a	6.2 a	33.38 a	31.11 b
(g/tree)	96	64.06 a	3.7 a	33.73 a	37.91 a

Table 2. Effects of Iron and nitrogen on L*, a*, b* and Total antioxidants in "Red spur "cultivar.

Within each column, means followed by the same letters are not significantly different by the duncan test at $P \le 0.05$.

Table 3. Effects of Iron and nitrogen on fruit firmness, TSS, TA and TSS/TAin "Granny smith" cultivar.

Treatme	nts	Fruit Firmness (N)	TSS (%)	TA (%)	TSS/TA
Luon	0	77.1 a	10.83 b	0.729 b	15.11 b
Iron	500	76.2 b	11.20 a	0.713 c	16.03 a
(mg/tree)	1000	74.8 c	10.7 b	0.737 a	14.96 b
NT	0	76.7 a	11.15 a	0.713 b	15.76 a
Nitrogen	48	77.0 a	10.69 c	0.732 a	15.12 a
(g/tree)	96	74.8 b	10.89 b	0.732 a	15.23 a

Within each column, means followed by the same letters are not significantly different by the duncan test at $P \le 0.05$.

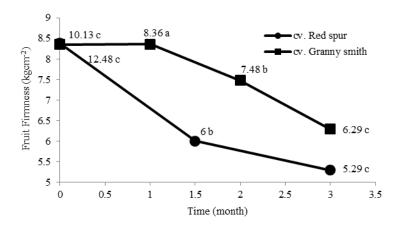


Fig. 1. Changes in fruit firmness of two apple cultivars during storage at 4±0.5 °C.

TSS, TA and TSS/TA

The total soluble solid in "Red spur" cultivar was increased during storage from 12.48 % for fresh harvested fruit to 14.59 % in fruit stored for 90 days. During storage period of "Granny smith" cultivar, increasing trend up to the third stage was observed, and thereafter declined at the end of storage (Fig. 2, A). According to the Table 1, in "Red spur" cultivar, TSS was significantly decreased by 1000 mg Iron application, while, 48 g/tree N significantly increased it. While, in "Granny smith" cultivar, Iron treatment in 500 mg

increased TSS and N nutrition decreased it (Table 3). The lowest value of TSS was observed in 48 g/tree (10.69 %).

As it is evident from Figure 2, B, continuous decreasing in TA percentage during the storage period was observed in both cultivars. In this investigation use of Iron nutrition in 500 mg increased TA and 1000 mg Iron application decreased it. N application has no effect on TA (table 1). In "Granny smith" cultivar, 500 mg Iron treatment decreased TA and 1000 mg increased it. N in both levels also significantly enhanced TA (Table 3).

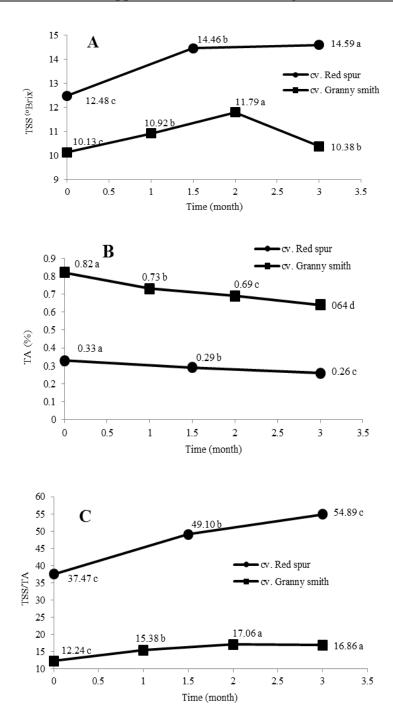


Fig. 2. Changes in TSS (A), TA (B) and TSS/TA (C) of two apple cultivars during storage at 4±0.5 °C.

With regards to Figure 2, C, there was continuous increasing in TSS/TA in both cultivars during the storage period. In the present study, TSS/TA was significantly increased by using of 1000 mg Iron in "Red spur" cultivar and 500 mg in "Granny smith" cultivar (Table 1 and 3). In both cultivars, N treatments have no effect on TSS/TA (table 1 and 3).

Total antioxidants

In the current study, in "Red spur" cultivar total antioxidant capacity was increased at the end of storage (Fig. 4) and using of Iron has no effect on its content, while application of 48 g/tree N resulted in lower antioxidant content (Table 2). In "Granny smith" cultivar, Iron and N application have no effect on antioxidant content (Table 4).

Nitrogen

(g/tree)

0

48

96

Treatmen	nts	L*	a*	b *	Total antioxidants
T	0	64.46 a	-19.63 a	38.15 c	34.12 a
Iron	500	64.63 a	-19.87 a	39.0 a	31.3 a
(mg/tree)	1000	64.62 a	-19.8 a	38.58 b	30.03 a

-19.68 a

-19.72 a

-19.89 a

38.65 a

38.49 a

38.58 a

33.77 a

31.87 a

29.77 a

Table 4. Effects of Iron and nitrogen on L*, a*, b* and Total antioxidants (%) in "Granny smith" cultivar

Within each column, means followed by the same letters are not significantly different by the duncan test at $P \le 0.05$.

64.88 a

64.44 b

63.39 b

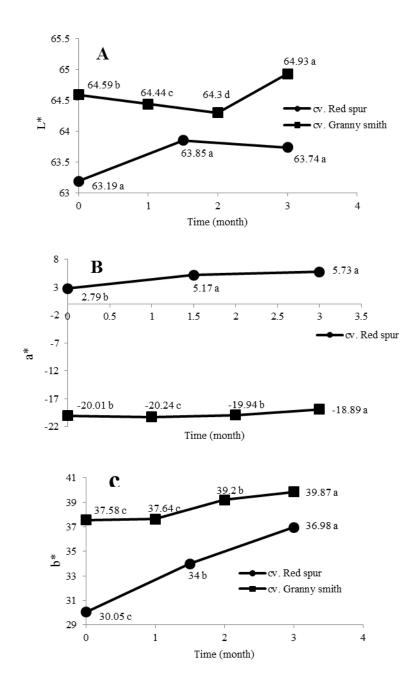


Fig. 3. Changes in L* (A), a* (B) and b* (C) of two apple cultivars during storage at 4±0.5 °C.

Parameters related to color

According to Figure 3, A, in "Granny smith" cultivar, a decreasing trend up to the third stage was observed for L* parameter and thereafter it was increased at the end of storage. There was no significant change for this parameter in "Red spur" cultivar. In "Granny smith" cultivar a decreasing trend up to the second stage was observed for a* parameter and then it was increased up to end of storage (Fig. 3, B), meanwhile, in "Red spur" cultivar this value was significantly increased with the increase in storage time. On the other hand, there was continuous increase in b* parameter in both cultivars during the storage (Fig. 3, C).

As it is evident from Table 2, in cultivar "Red spur", 500 mg Iron treatment significantly enhanced a* and decreased b* parameter. No changes were observed either in the color parameters by N treatments in this cultivar. In cultivar "Granny smith", b* parameter was influenced by Iron and L* parameter was affected by N application. According to table 4, Mean value of L* parameter was significantly lower in both N treatments compared with control and Iron treatments significantly increased b* parameter. The highest value of b*was recorded in 500 mg (39.00).

Table 5. Effects of interaction between Iron and nitrogen on some qualitative traits in cultivar "Red spur" and "Granny smith".

Treatments		Red spur		Granny smith			
Iron (mg/tree)	Nitrogen (g/tree)	TSS (°Brix)	TSS/TA	L*	TA (%)	TSS/TS	
	0	13.43 e	44.17 d	64.87 b	0.687 d	16.49 a	
0	48	15.00 a	50.77 a	64.37 cd	0.738 b	14.7 c	
	96	13.45 e	43.72 d	64.16 e	0.760 a	14.13 d	
	0	14.04 c	47.56 c	64.52 c	0.744 b	15.74 b	
500	48	13.64 d	43.08 d	64.54 c	0.717 c	15.66 b	
	96	14.49 b	47.17 c	64.81 b	0.677 e	16.69 a	
1000	0	13.72 d	50.60 a	65.24 a	0.708 c	15.05 c	
	48	13.73 d	48.22 bc	64.41 c	0.744 b	14.97 c	
	96	13.06 f	49.09 b	64.19 de	0.760 a	14.87 c	

Within each column, means followed by the same letters are not significantly different by the duncan test at $P \le 0.05$.

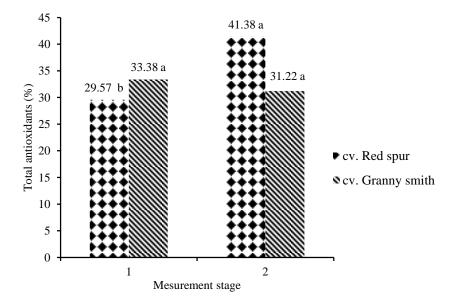


Fig. 4. Changes in total antioxidants of two apple cultivars at the start and end of storage at 4 ± 0.5 °C.

Discussion

In the current study, quality of apples fruit was changed during the storage time. Changes in fruit quality can be investigated physical and chemical analyses depending on storage time. Loss of chemical ingredients and texture are important problems in stored apples (Kov and Felf, 2003). The loss of fruit firmness during storage is a serious concern as it results in quality losses leading to soft and mealy fruit and hence affect consumer demands (Jan et al., 2012). Decrease in fruit firmness during storage has been frequently reported and is caused by various factors that mentioned as following. The fruit firmness depends on the texture of the flesh and changes in primary cell wall during ripening and it may involve disassembly of primary cell wall and middle lamella structures due to enzymatic activities and pectin solubilization (Jan et al., 2012). Enzymatic cell wall breakdown mainly affects the mechanical strength of the tissue (Roth et al., 2005). The responsible enzyme for pectin solubilization in most fruits is polygalacturonase) Johnston et al., 2002). Accumulation of ethylene could facilitate the mRNA translation of degradation enzymes, followed by the degradation of cell wall polysaccharides and fruit softening (Chang-Hai et al., 2006). On the other hand, water loss due to transpiration causes reduced turgidity of the cells and mainly affects the stiffness of intact fruit (Roth et 2005). As mentioned above Iron application decreased fruit firmness in both cultivars .This result likely related to ripening time .A delay in fruit ripening has been reported to occur with Fe deficiency in some fruits) Álvarez-Fernández et al... .Nitrogen fertilization has been 2003) shown to result in a decrease in firmness of strawberry (Miner et al., 1997) and apple (Nava et al., 2008). This undesirable decrease in firmness due to excess nitrogen fertilization is well documented for several crops (Sams, 1999). This effect is usually associated with calcium .Nitrogen promotes

vegetative growth which drives more Calcium towards the leaves and thereby reduces the fruit Calcium concentration (Nava and Dechen, 2009). Positive effects of calcium on fruit firmness and positive correlation between them have been frequently reported (Casero et al., 2004).

Total soluble solids (TSS), titratable acidity (TA) and the ratio of TSS/TA are important factors for evaluating fruit quality (Kafkas et al., 2007). One of the most important quality attributes that directly influence consumers on purchasing fresh apple fruit is sugar content (Lu, 2004). Increasing of TSS during storage is likely due to decline of starch content and the hydrolysis of complex cell wall polysaccharides into simple sugars (Jan et al., 2012). An increase in TSS during storage reported in apple (Jan et al., 2012) and orange fruit (Rapisarda et al., 2008). The decrease in TSS content at the end of storage might be due to increase in respiration rate and conversion of sugars to carbon dioxide and H2O (Ghasemnezhad et al., 2010). TSS/TA was continuously increased in both cultivars during the storage period. Similar result was also reported in orange (Rapisarda et al., 2008) and peach fruit (Infante et al., 2008).

Iron chelate application increased glucose and TSS in peach (Hasna and Mustapha, 2014), which is concordant to the results observed in the "Granny smith" cultivar. Increase of TSS by Iron application is probably due to its effect on the leaf pigments and photosynthesis that cause higher carbohydrate production (Terry, 1980). Davarpanah et al. (2013) similarly found that soil application of Iron increased the TSS/TA ratio in pomegranate. On the increases in carboxylate other hand, concentrations and decreases sugar/organic acid ratio under Fe chlorosis condition was reported in peach fruit (Alvarez-Fernandez et al., 2011). Nitrogen fertilization in orange increased the soluble solids content (Quaggio et al., 2006), another study reported that application of

low nitrogen level compared to high levels led to increase in TSS and fructose in apple (Raese and Drake, 1997) which is similar to the results observed in the "Red spur" cultivar. Decline in TSS of Apple fruit is also reported by nitrogen application during storage (Noe et al., 1997).

Acidity plays an important role in the determination of fruit quality. It affects not only the sour taste of the fruit, but also sweetness, by covering the taste of sugars (Lobit et al., 2003). The predominant organic acid in ripe fruit varies among species. In apple fruit malic acid is the dominant acid (Etienne et al., 2013). The titratable acidity content of the fruit depends on the rate of metabolism especially respiration which consumed organic acid and thus decline acidity (Jan et al., 2012). Nitrogen fertilization may have an indirect impact on fruit acidity by stimulating the vegetative growth of plants .Increased vegetative growth may affect fruit quality by shading them) which decreases their temperature and reduces transpiration), or by diverting assimilates towards vegetative growth which reduces the supply of assimilates to the fruits (Etienne et al., 2013).

Antioxidants are substances that have a very important role in strengthening the human immune system (Murtić et al,. 2013). These compounds can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Javanmardi and Kubota, 2006). Fruit crop contain high antioxidant capacity (Wang, 2006). Phenolic compounds are the primary molecules responsible for the antioxidant capacity of fruits (Hoang et al., 2011). The quantity and quality of antioxidants in fruits could be improved through the selection of different fruit cultivars and improved preharvest conditions (Wang, 2006). The synthesis of antioxidant substances in the plant is closely related to the photosynthetic process; therefore, different agro-technical practices including fertilization that are used

in apple cultivation can increase the rate of photosynthesis) Murtić et al., 2013) .Storage also influence phytochemical can composition of food crop (Wang, 2006). The variability in the phenolic compounds during storage, have been reported in some studies that may due to many factors including apple cultivar, storage conditions and/or postharvest treatments (Hoang et al., Increasing antioxidant activity during low temperature storage could be related to the ripening processes and phenolic metabolism of compounds (Javanmardi and Kubota, 2006). Significant increase in antioxidant activity of tomatoes stored in cold storage has been reported (Javanmardi and Kubota, 2006). Similar results were observed in apples (Hoang et al., 2011) and oranges (Hamedani et al., 2012). Porto et al. (2016) found that increasing the N concentration also reduced the content of bioactive compounds, which consequently impaired antioxidant activity in tomato plant (Porto et al., 2016). Nagy (1980) have reported the reduction of ascorbic acid content in citrus fruits by increasing the nitrogen supply. However, more research is needed to demonstrate the effect of N on the antioxidant activity of apple (Porto et al., 2016).

Fruit skin color is an important quality parameter which determines consumer acceptability of fruits like apple (Dixon and Hewett, 1998). It depends on many harvesting and postharvest factors(Ganai et al., 2015). Color development, is in principle genetically determined, however production and environmental factors play a significant role (Kuhn et al ,.2011). Storage influences the final quality of fruit, as it affects the appearance and induces color change (Dobrzański and Rybczyński, 2002). Anthocyanins are responsible for the red pigmentation in apple peel (Hoang et al., 2011). Production and environmental factors play a significant role in upregulation of anthocyanin level (Kuhn et al., 2011). It has been reported that Fe deficiency caused decreases in the mean a* color coordinate and increases in the mean L* and b* color coordinates color of peach fruit (Álvarez-Fernández et al., 2003). The decrease in total photosynthate production caused by Fe deficiency maybe one of the reasons for the less intense red color observed (Álvarez-Fernández et al., 2011). On the other hand, Anchondo et al. (2001) positive observed and significant correlation between leaf Iron and redness of fruit. Many studies have demonstrated a negative relationship between N-supply and fruit color (Fallahi et al., 2001; Raese, 1998; Fallahi and Mohan, 2000). In a study done by (Ganai et al., 2015) there was continuous increase in L* and b* values and decrease in a* values of apple during the storage period. Viškelis et al. (2011) observed fruit color parameters like L*, a*, b* were increased after eight months of normal cold storage. These changes in color parameters might be the pigment degradation during the storage (Ganai et al,. 2015). The results of present study are in agreement with the results of other investigators that mentioned above.

In this study, soil application of Fe and N on two apple cultivars during cold storage was evaluated. The results showed that in "Red spur" cultivar, Fe application decreased fruit firmness, TSS and b* parameter value and increased TSS/TA and parameter while, N application TSS. Fruit firmness increased decreased and TSS. TSS/TA and b* parameter were increased by Fe application in "Granny smith" cultivar. In this cultivar fruit firmness, TSS and L* parameter values were decreased and TA was increased by N treatments. In conclusion, preharvest application of Fe and N in proper levels improved postharvest qualitative characteristics in both apple cultivars during cold storage.

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