Effects of Pre-Harvest Methyl Jasmonate Treatments on Fruit Quality of Fuji Apples during Cold Storage

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Keywords:	Abstract. This study was conducted to identify the effects of different doses (1120, 2240 and 4480		
Weight loss, <i>Malus x</i> <i>domestica</i> , starch index,	mg L ⁻¹) of methyl jasmonate (MeJA) treatments on apple fruit quality at the cold storage. In the study,		
firmness, SSC, titratable	5 year-old Fuji apple trees grafted on M9 rootstock were used. MeJA was applied to the trees three		
acidity	different times (2, 3 and 4 weeks before the commercial harvest date). It has been determined that the		
	effect of MeJA treatment on weight loss during cold storage has changed depending on the		
	concentration of MeJA. With the increase in application dosage of MeJA, the fruit fresh softening		
	significantly delayed. The fruits treated with MeJA have higher soluble solids content (SSC) and		
*Corresponding author	titratable acidity during cold storage. While the fruits treated with MeJA had lower pH values, no		
erdalaglar@hotmail.com	significant effect of MeJA treatment on starch index was observed. As a result, it was determined that		
	fruit flesh firmness loss that occurs during cold storage can be delayed by MeJA treatment.		

Hasat Öncesi Metil Jasmonat Uygulamalarının Soğukta Muhafaza Süresince Fuji Elmasının Meyve Kalitesi Üzerine Etkisi

Anahtar kelimeler: Özet. Araştırma hasat öncesi farklı dozlarda uygulanan (1120, 2240 ve 4480 mg L⁻¹) metil jasmonatın Ağırlık kaybı, *Malus* Х (MeJA) soğukta muhafaza süresince Fuji elmasının meyve kalite özellikleri üzerine olan etkilerini domestica, nişasta indeksi, belirlemek için yürütülmüştür. Araştırmada, M9 anacı üzerine aşılı 5 yaşlı Fuji elma ağaçları kullanılmıştır. sertlik, SÇKM, titre edilebilir MeJA, 3 farklı zamanda (ticari hasattan 2, 3, ve 4 hafta önce) ağaçlara uygulanmıştır. Soğukta muhafaza süresince ağırlık kaybı üzerine MeJA'nın etkisinin uygulama dozuna bağlı olarak değiştiği tespit edilmiştir. MeJA uygulama dozu arttıkça, meyve etinde meydana gelen yumuşama önemli derecede geciktirilmiştir. Depolama süresince, MeJA ile muamele olan meyvelerin SÇKM ve asitlik içeriği daha yüksek bulunmuştur. MeJA ile muamele olmuş meyvelerin pH değeri düşük olmasına rağmen, nişasta indeksi üzerine MeJA uygulamalarının önemli bir etkisi gözlemlenmemiştir. Sonuç olarak, soğukta muhafaza süresince meyve eti sertliğinde meydana gelen kaybın MeJA uygulaması ile geciktirilebileceği belirlenmiştir.

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INTRODUCTION

Apple is one of the fruit species that have the highest world production. Due to its fruit qualities and the fact that it can be cultivated in many regions of Turkey, apple is prefered and consumed by the consumers throughout the year. Consumption of apples year round is possible with high-quality fruits at harvest and post-harvest storage. As with many fruit species, it is very important to maintain post-harvest quality of apple and to provide continuous supply in the market. Apple can be stored for a longer time due to its fruit characteristics. But it is not possible to maintain fruit quality as it is untill the end of cold storage. In this sense, it is important to minimize fruit quality losses that occur during cold storage.

The quality losses, which occur at the cold storage, can be reduce by the preharvest (Zhang and Whitting 2011; Einhorn *et al.*, 2013; Gimenez *et al.*, 2014; Martinez-Espla *et al.*, 2014; Valverde *et al.*, 2015) and postharvest (Petracek *et al.*, 2002; Valero *et al.*, 2011; Giacalone and Chiabrando 2013; Valero *et al.*, 2014) applications.

The pre-harvest treatments improve fruit quality and have positive effects at the cold storage, while post-harvest treatments prevent potential losses in fruits during cold storage (Meheriuk *et al.*, 1995).

Due to the development of resistance by pathogens to some fungicides and food safety and environmental pollution, the alternative measures was developed in controling of diseases at cold storage (Ragsdale and Sisler 1994). Therefore, pre-harvest treatments that can increase fruit firmness and protect fruit against diseases that may occur after harvest are in the foreground.

Previous researchers tried to reduce these postharvest losses with different growth regulators like aminoethoxyvinylglycine (Öztürk et al., 2017), gibberellic acid (Ozkan et al., 2016), methyl salicylate (Valverde et al., 2015), lysophosphatidylethanolamine (LPE) (Özgen et al., 2015), salicylic acid (Gimenez et al., 2015, 2017), Aloe vera (Castillo et al., 2010) and methyl jasmonate (Kucuker and Ozturk 2015). Methyl jasmonate (MeJA), elicitor or signalling agent involved in many physiological and biochemical processes, exists naturally in a wide range of higher plants (Creelman and Mullet 1997). It has been reported that postharvest MeJA treatment has reduced the number of stress-induced injuries during cold storage, such as chilling injury, mechanical and salt stress (Pena-Cortes et al., 2005; Sayyari et al., 2011).

It has been determined that MeJA treatment suppressed fungal decay, for example, grey mold rot in strawberry (Moline *et al.*, 1997), and anthracnose in tomato (Tzortzakis 2007) and loquat fruit (Cao *et al.*, 2008). Yao and Tian (2005) have determined that preharvest MeJA treatments reduce fungal infections caused by Monilinia fructicola on sweet cherry. It has also been reported that the pre-harvest MeJA treatment delayed the softening of fruit during storage (Rudell *et al.*, 2005). The studies with MeJA generally focus on postharvest treatments and the fruit firmness mechanism.

There are very few studies on pre-harvest MeJA treatments and their effects on harvested and postharvest storage fruit quality. In this study, which had been carried out by considering this situation, the effects of pre-harvest MeJA treatments on the fruit quality during harvest and after harvest and on losses occur at cold storage have been evaluated.

MATERIALS AND METHODS

Plant Material and Experimental Design

The study carried out with five year-old Fuji trees grafted on M9 rootstock was laid out as three replicates according to randomized plots trial design and there were 4 trees in each replicate. Cultural practices such as irrigation, fertilization, pruning and thinning were carried out on the trees. The trees were trained as slender spindle training system. Irrigation was carried out with double-line drip irrigation system with plumbing irrigation pipes positioned at 20 cm intervals giving 2 liters of water per hour. The study consisted of four treatments: three different concentrations of MeJA (1120, 2240 and 4480 mg L⁻¹) and control (0 mg L⁻¹). The treatments of MeJA were applied repetitively at three different times: 2, 3 and 4 weeks before commercial harvest date. During the commercial harvest, 10 kg of fruit was harvested for each repetition and 3 kg of fruit (1 kg for each repetition) was allocated for each analysis period. The fruit was harvested by hand and injured, diseased or harmed fruits were removed.

Besides measurements made during harvest, additional measurements were performed in 2^{nd} , 4^{th} and 6^{th} months. All fruits were stored at 0°C and $90\pm5\%$ RH for 6 months in the same storage which has normal atmosphere conditions, and quality analysis and measurements were made in 60-days intervals.

Weight Loss

Fruit weights were determined using a digital scale $(\pm 0.01 \text{ g})$ (Radvag PS 4500/C/1, Poland). Weight loss was determined by the difference between the initial and final weights of each replicate during cold storage and expressed as percent.

Fruit Flesh Firmness

After the fruit peel has been removed by a cutting tool, flesh firmness (five fruits) was measured on three sides of equatorial line of each fruit using a pressmounted penetrometer (FT-327 MoCormick Fruit Tech., Yakima, ABD) with 11.1 mm tip. The values measured are expressed as Newton (N).

Solible Solids Content, pH and Titratable Acidity

A sample of juice was taken from one piece of each of ten fruits per tree, and 3 different measurements were obtained from each replication. SSC was determined with a digital refractometer (PAL- 1, McCormick Fruit Tech., Yakima, Wash) and the values had been expressed as % (°Brix). For titratable acidity (TA), 10 mL of extract was taken from each sample, 10 mL distilled water was added and the value corresponding to consumed sodium hydroxide (NaOH) during the titration with 0.1 N NaOH to increase the pH of samples to 8.1 was expressed in malic acid (g malic acid 100 mL⁻¹).

Starch Index

Samples whose fruit firmness was measured were divided into 2 equal parts and a circular slice about 1 cm thick was taken from the remaining part of the stem side and 0.5% iodized potassium iodide (IKI) solution was sprayed to the circular slice until it was completely wetted.

After about 5 minutes, the area containing the starch was stained dark blue and values were given according to the scales (1-8 scale interval, 1 = 100% blue painted, 8 = 100% starch turned into sugar) that the investigators (Blanpied and Silsby 1992) had prepared.

Statistical Analysis

The experiment was set up in 3 replications according to randomized plot trial design and there were 4 trees in each repetition and 48 trees were used in total. All statistical analyses were performed with SAS Version 9.3 (SAS Institute Inc., Cary, NC, USA). Data were analyzed by analysis of variance. Mains of treatments were separated by Duncan's multiple range tests at a significance level of 0.05.

RESULTS

Weight Loss

Considering the storage times, the fruit weight loss increased in proportion to the cold storage time. It has been determined that the effect of MeJA on weight loss depends on concentration and cold storage times. On the 60th day of the cold storage, while 4480 mg L⁻¹ MeJA treatment did not affect weight loss, 1120 mg L⁻¹ ¹ MeJA treatment led to an increasing in weight loss and 2240 mg L⁻¹ MeJA treatment led to a decrease in weight loss. On the 120th and 180th day of storage, it has been observed that weight losses in control and 2240 mg L⁻¹ MeJA treatment were at the similar levels, whereas 1120 mg L⁻¹ MeJA and 4480 mg L⁻¹ MeJA doses increased weight loss (Table 1).

 Table 1. Effects of pre-harvest MeJA treatments on weight loss of Fuji apples.

Çizelge 1. Fuji elmasının ağırlık kaybı üzerine hasat öncesi MeJA uygulamalarının etkisi.

MalA treatments	Weight loss (%)				
weja treatments	60	120	180		
Control (0 mg L ⁻¹)	0.98 b	1.53 b	2.15 b		
1120 mg L ⁻¹ MeJA	1.53 a	2.40 a	3.27 a		
2240 mg L ⁻¹ MeJA	0.65 c	1.44 b	2.09 b		
4480 mg L ⁻¹ MeJA	1.16 b	2.17 a	2.98 a		

The means with the same letter do not differ according to Duncan's multiple range test, P < 0.05.

Fruit Flesh Firmness

It has been observed that with the MeJA dose increases, the softening of fruit flesh delayed. It was determined that the increase in cold storage duration caused a decrease in the flesh firmness values in all treatments. The maximum decrease in fruit firmness values at the end of the storage was found to be in the control treatment. It has been determined that MeJA treatment has a significant effect on maintaining fruit firmness at cold storage and the effect of MeJA treatment changes depending on the concentration and the effectiveness increases with increases in application concentrations (Table 2).

Solible Solids Content (SSC)

Fruits with high SSC ratio were harvested from MeJA treated trees, but the difference in SSC at harvest between MeJA dose was not significant. Considering the cold storage durations, it was determined that the rates of SSC increased up to 120 days, and then decreased. While the MeJA application is effective in changes in SSC in storage, this effect causes the values to decrease at the 60th and 120th days of storage and increase at the end of storage (Table 3).

T a Çi	Table 2. Effects of pre-harvest MeJA treatments on fruit flesh firmness of Fuji apples.Çizelge 2. Fuji elmasının meyve eti sertliği üzerine hasat öncesi MeJA uygulamalarının etkisi.					
-			J	Fruit flesh f	irmness (N)	1
	MeJA tre	atments -	Harvest	60	120	180
-	Control	(0 mg L ⁻¹)	69.45 c	67.65 c	66.38 c	52.32 c
	1120 mg	L ⁻¹ MeJA	77.69 b	76.85 b	71.12 b	69.32 b
	2240 mg	L ⁻¹ MeJA	84.56 a	82.08 a	69.00 b	68.34 b
	4480 mg	L ⁻¹ MeJA	86.79 a	84.37 a	77.17 a	75.21 a

The means with the same letter do not differ according to Duncan's multiple range test, P<0.05.

Table 3. Effects of pre-harvest MeJA treatments on SSC ofFuji apples.

Çizelge 3. Fuji elmasının SÇKM içeriği üzerine hasat öncesi MeJA uygulamalarının etkisi.

MeJA treatments	SSC (%)			
	Harvest	60	120	180
Control (0 mg L ⁻¹)	13.7 a	14.7 a	15.5 a	13.1 b
1120 mg L ⁻¹ MeJA	13.0 b	14.1 b	14.4 b	13.7 a
2240 mg L ⁻¹ MeJA	13.1 b	13.2 c	15.8 a	13.8 a
4480 mg L ⁻¹ MeJA	12.9 b	14.0 b	14.6 b	13.7 a

The means with the same letter do not differ according to Duncan's multiple range test, P < 0.05.

Titratable Acidity

When the values of measurable acidity measurements after harvest and during storage were examined, it was determined that there was no statistically a significant difference between control and treatment of 1120 mg L⁻¹ MeJA at harvest and at 60th and 120th days of storage, and only at the 180th day of cold storage a difference was observed. However, with MeJA treated at concentrations of 2240 mg L⁻¹ and 480 mg L⁻¹, it was determined that the titratable acidity values were increased and the acid content at cold storage was maintained. As a result of the analysis during cold storage period, the increase in the duration cold storage leads to a decrease in the ratio of titratable acidity (Table 4).

Table 4. Effects of pre-harvest MeJA treatments on titratabale acidity content of Fuji apples.

Çizelge 4. Fuji elmasının titre edilebilir asitlik içeriği üzerine hasat öncesi MeJA uygulamalarının etkisi.

MalA treatments	Titratabale acidity (% malic acid)			
MeJA treatments	Harvest	60	120	180
Control (0 mg L ⁻¹)	0.42 b	0.37 b	0.31 b	0.23 b
1120 mg L ⁻¹ MeJA	0.41 b	0.39 b	0.30 b	0.27 a
2240 mg L ⁻¹ MeJA	0.48 a	0.45 a	0.35 a	0.30 a
4480 mg L ⁻¹ MeJA	0.52 a	044 a	036a	029a

The means with the same letter do not differ according to Duncan's multiple range test, P<0.05.

pН

When it was compared to the control treatment, it has been determined that the pH values obtained at - the harvest or at the end of the cold storage were _ lower in the fruits treated with MeJA. It has been found _ that an increase in the pH values of all treatments in proportion to the cold storage durations occurred and that increase was higher in the control treatment (Table 5).

Table 5. Effects of pre-harvest MeJA treatments on pH value of Fuji apples.

Çizelge 5. Fuji elmasının pH değeri üzerine hasat öncesi MeJA uyqulamalarının etkisi.

	рН			
weja treatments	Harvest	60	120	180
Control (0 mg L ⁻¹)	3.96 a	4.06 b	4.38 a	4.79 a
1120 mg L ⁻¹ MeJA	3.87 b	4.07 b	4.26 b	4.51 b
2240 mg L ⁻¹ MeJA	3.85 b	4.18 a	4.29 b	4.52 b
4480 mg L ⁻¹ MeJA	3.87 b	4.16 a	4.28 b	4.56 b

 y 1 = 100% starch and 8 = 0% starch. The means with the same letter do not differ according to Duncan's multiple range test, P<0.05.

Starch Index

In the analyses made at harvest, the effect of 1120 mg L⁻¹ MeJA on starch index was not significant, whereas with 2240 mg L⁻¹ MeJA and 4480 mg L⁻¹ MeJA, the amount of starch decreased. Retention of fruits in storage has led to an increase in the starch index. It has been determined that there were significant differences in starch index values between treatments on the 60th day of storage. The highest value was obtained by application of 2240 mg L⁻¹ MeJA, while the lowest value was recorded with 1120 mg L⁻¹ MeJA. However, no statistically significant difference was found between 4480 mg L⁻¹ MeJA and control treatments. On the 120th and 180th days of cold storage, it had been determined that there was no difference between the treatments in terms of starch quantities (Table 6).

 Table 6. Effects of pre-harvest MeJA treatments on starch index of Fuji apples.

Çizelge 6. Fuji elmasının nişasta indeksi üzerine hasat öncesi MeJA uygulamalarının etkisi.

MeJA treatments	Starch index ^y			
	Harvest	60	120	180
Control (0 mg L ⁻¹)	6.0 a	7.0 b	8.0 a	8.0 a
1120 mg L ⁻¹ MeJA	6.2 a	6.3 c	8.0 a	8.0 a
2240 mg L ⁻¹ MeJA	5.5 b	8.0 a	8.0 a	8.0 a
4480 mg L ⁻¹ MeJA	40 c	73b	80a	80a

 y 1 = 100% starch and 8 = 0% starch. The means with the same letter do not differ according to Duncan's multiple range test, P<0.05.

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DISCUSSION

After harvest, the weight loss due to water loss in fruit leads to significant economic losses by affecting the structure and the appearance of the fruit. In the study we have done to minimize the losses that may occur at cold storage, it was determined that the weight loss of the fruit was increased during cold storage. Similar to our results, Veravrbeke et al. (2003) found that an increase in postharvest cold storage duration increased the weight loss of the fruit, and that the loss varied depending on the amount of waxy layer on the fruit surface and chemical structure of the fruit. Gavalheiro et al. (2003) reported that the waxy layer could maintain its strength until the 90th day after harvest, and that the wax layer was damaged and the losses increased after 120 days of cold storage. In the study which was conducted with an expectation that MeJA may enhance the resistance mechanism of the plant and contribute to the amount and chemical content of the wax layer, it was observed that MeJA treatment has not been effective in preventing weight loss at cold storage. Fujita et al. (2006) and Wasternack (2007) reported that jasmonates (methyl jasmonate and jasmonic acid) are multifunctional compounds that play a role in critical processes associated with biotic and abiotic stress response as well as plant development. Jasmonates are able to elicit protective effects against the plant stress by reducing hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) levels and increasing antioxidant capacity in plants (Maksymiec and Krupa 2002; Piotrowska et al., 2009; Kovacik et al., 2011; Dar et al., 2015). Hentrich et al. (2013) have found that jasmonates promote auxin (IAA) biosynthesis. Fruit firmness is the most significant fruit quality characteristic that is effective at cold storage.Quality fruit is the first condition for maintaining fruit firmness at cold storage, but it is impossible to maintain the fruit quality obtained at harvest until the end of cold storage. The storage period can be extended by minimizing fruit firmness loss by preharvest and postharvest applications. In the study, which was carried out towards this aim, higher quality fruit at harvest has been achieved by preharvest MeJA treatment. It was determined that MejA was effective in maintaining fruit firmness at cold storage, the effect of MeJA treatment changes depending on the concentration and the increase in concentration also increased the effectiveness. Karaman et al. (2013) reported that MeJA applied 2 weeks before harvest did not affect the fruit firmness, but after 28 days of the cold storage MeJA treated plums remained firmer than controls. (Martinez-Espla *et al.*, 2014), has determined that preharvest MeJA treatments resulted in firmer fruits at time of harvest in 'Black Splendor' and 'RoyalRosa' plums.

Methyl jasmonate (MeJA) is a natural plant growth regulator with a great role played in plant growth, development and resistance to environmental stressors (Rudell et al., 2002; Kondo 2005). MeJA has significant effects on physical (size, color, firmness) and chemical (pH, SSC, TA) characteristics and pigment contents of fruits (Ozturk et al., 2013). JAs treatments positively effect fruit quality characteristics in many fruit (Rohwer and Erwin 2008). Perez et al. (1993) reported that JA stimulated carotene synthesis in tomato, and (Rudell and Mattheis 2008) have determned that exgenous JAs led to phenolic compound (anthocyanin) accumulation. Furtermore, JAs promoted volatile emission in apple (Kondo et al., 2005) and has an effect on fruit quality and antioxidant capacity in blackberry (Wang et al., 2009). Martinez-Espla et al. (2014), have reported that preharvest MeJA treatments led to fruit with higher size and weight, firmness and TA at time of harvest in 'Black Splendor' and 'RoyalRosa' plums, and the phenolic content and antioxidant activity were also higher in treated than in control fruit during the last 3 weeks of fruit development in both plum cultivars.

In the study, in which fruits with higher ratio of SSC were harvested by MeJA treatment, MeJA has had an effect on changes that occur in SSC at cold storage. It was determined that MeJA treatment increased the titratable acidity values and had an effected on maintaining the acid content at cold storage. It has been determined that the effect of MeJA on the starch index is not significant, and pH values were lower in MeJA treatment.

CONCLUSION

As a result of the study, MeJA application significantly reduced the quality losses at cold storage. With MeJA treatment, the softening of fruit is delayed significantly. As a result, it is determined that the fruit firmness loss that occurs during cold storage can be delayed by MeJA treatment.

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