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Possible chemical mechanism and determination of inhibitory effects of 1-MCP on superficial scald of the Granny Smith apple variety

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Abstract: The effects of postharvest 1-methylcyclopropene (1-MCP) treatments on α -farnesene content, incidence of scald, and ethylene content of the Granny Smith apple were evaluated in this study. Harvested apples were treated with 1-MCP at concentrations of 312.5, 625, and 1250 ppb. The 625 and 1250 ppb doses were found to be the most effective in terms of quality protection during storage. For untreated fruits, symptoms of scald development appeared after 8 weeks in cold storage, whereas 1-MCP doses of 625 and 1250 ppb were effective in decreasing superficial scald in fruit after 24 weeks of cold storage. 1-MCP can inhibit oxidation of α -farnesene, which synthesizes during storage. This induces reactive oxygen species metabolism. 1-MCP introduces competitive behavior by breaking the balance between α -farnesene production and the antioxidative defense system, due to the Schenck ene reaction.

Key words: α-Farnesene, Granny Smith apple, 1-methylcyclopropene, Schenck ene reaction, superficial scald

1. Introduction

Granny Smith is the third most important apple cultivar grown in Turkey. Moreover, Turkey, producing over 2.5 million tons of apples annually, is one of the most important apple-producing countries in the world (FAO, 2002). In recent years, the Granny Smith apple variety has become increasingly important, after the Starking Delicious and Golden Delicious apple varieties, because of high consumer demand in Turkey. It is often superior to other apple cultivars in terms of quality and taste property. During the winter season, Granny Smith apples sold in shops and markets are obtained from cold storage facilities. Superficial scald development on Granny Smith apples is a common problem often found in cold storage. Apple cultivars vary greatly in susceptibility to scald, e.g. Granny Smith, Law Rome, Fuji, and Delicious are susceptible, while Empire, Gala, Braeburn, Pink Lady, and Golden Delicious are found to be resistant (Lurie and Watkins, 2012). Superficial scald is a physiological disorder of apples and pears and its development has been associated with naturally occurring volatile sesquiterpene α-farnesene (Huelin and Coggiola, 1970).

 α -Farnesene is a chemically unstable volatile compound of the terpene group that can easily be oxidized in the presence of oxygen. α -Farnesene oxidation in fruit

cuticles and scald progress in apples are slow processes (Ju and Bramlage, 1999). Concentrations of α -farnesene in apples are quite low at the preclimacteric stage. However, they increase during ripening due to rising ethylene concentrations (Barden and Bramlage, 1994). It usually takes 2 or 3 months for a significant decrease in α -farnesene accumulation to occur and at least 3 months for scald.

Antioxidants such as AVG (aminoethoxyvinylglycine), DPA (diphenylamine), or EQ (ethoxyquin) repress the auto-oxidation of a-farnesene by inhibiting ethylene production and decreasing the intensity of the scald. However, the effectiveness of the application of DPA is questionable and the use of EQ has already been prohibited due to increased health concerns. Therefore, nonchemical approaches for preventing scald are currently being examined such as controlled atmosphere storage, especially at ultralow O₂ concentration, and heat treatments (Matich et al., 1998; Yazdani et al., 2011). However, chilling injury can intensify scald because chilling injury can increase polygalacturonase activity, damage to cell walls, and damage to organelles. Damaged cells are more sensitive to scald injury. Intermittent warming is another effective way to protect fruits from scald (Alwan and Watkins, 1999; Ju and Curry, 2000).

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Late harvesting also significantly reduces the development of superficial scald in Granny Smith apples (Erkan and Pekmezci, 2004).

Many factors, such as early harvesting, low temperatures under 10 °C, nutritional status of the fruit, fruit size, and storage temperature all have an effect on scald injury. 1-Methylcyclopropene (1-MCP) blocks ethylene (synthesis or sensation) and protects cells from the negative effects of ethylene (Sisler and Serek, 1997). It is a critical compound for extending the storage period of horticultural crops. 1-MCP blocks ethylene receptors and prevents ethylene effects on respiration, softening, and loss of titratable acidity for extended periods in apples. Moreover, it reduces volatile compound production by the stored apples (Fan et al., 1998; Watkins et al., 2000). Important factors for the efficacy of 1-MCP treatment are species, application time, treatment temperature, and type (powder, liquid, or gas) of the compound (Watkins, 2002).

There are important differences among the ethylene production of apple varieties. Early season varieties produce more ethylene than late season varieties. These varieties also show different responses to 1-MCP treatments (Watkins et al., 1989). 1-MCP treatments are not effective when climacteric respiration reaches a maximum level.

In this research, superficial scald, causing significant economic loss during cold storage of the Granny Smith apple, was examined by testing three different doses of 1-MCP treatment. The experiment was conducted in the Çanakkale region during the 2011–2012 storage seasons.

The aim of this study was to determine the effect of different doses of 1-MCP treatment on superficial scald and α -farnesene concentration of Granny Smith apples during cold storage. α -Farnesene concentration in the cold-stored Granny Smith apples was also investigated by gas chromatography-mass spectrometry (GC-MS) due to its role in superficial scald development.

2. Materials and methods

2.1. Fruit source

Nine-year-old Granny Smith (*Malus* × *domestica* Borkh.) apple trees, grafted onto Malling Merton 106 (MM106) rootstocks in a commercial orchard ($40^{\circ}24'04''N$, $26^{\circ}24'35''E$, 35 m above sea level) in Çanakkale, Turkey, were used as the plant material for this study. The trees were randomly selected from unblemished trees showing general characteristics of the apple cultivar. The fruits were carefully harvested by hand on 20 October 2012. The starch content of the harvested apples was determined by standard procedures using a starch index (1 to 8, Generic Starch Iodine Index Chart for Apples). When the average starch index reached 5, the apples were harvested. Only undamaged, commercial-size fruits (180–200 g) with specific color were used in this study.

2.2. 1-Methylcyclopropene treatments and storage

The 1-MCP treatments were carried out before storage on washed fruits on the day of harvest. 1-MCP Smartfresh was supplied by Agro Fresh (Rohm and Haas, Turkey) in protab form. Treatments of 1-MCP in doses of 312.5, 625 (used commercially on apples), and 1250 ppb were applied. The treatments were carried out for 24 h in a hermetically sealed container at 10–12 °C by means of an activator kit. Control fruits were kept at the same temperature for 24 h in a cold storage room.

This study was conducted in the cold storage rooms of the Department of Horticulture of the Faculty of Agriculture at Çanakkale Onsekiz Mart University. The treated and control fruits were stored at 0–1 °C and 90%–95% relative humidity (RH) in plastic boxes for 6 months. Storage conditions were also tracked using a TFA Data Logger (2H Ltd., Turkey).

2.3. Alpha farnesene (α-farnesene) content

The α -farnesene content of the stored apples was determined by GC–MS analysis followed by liquid–liquid extraction.

The liquid–liquid extraction was performed by use of diethyl ether solvent. Each extraction included two replications and each replication contained 100 g of apple puree obtained using a liquidizer (homogenizer). A volume of 100 mL diethyl ether solvent was added into an Erlenmeyer flask with 100 g of apple puree. After the solvent treatment, the extract was concentrated to 1 mL with a centrifuge and concentrator. Then the solvent was directly injected into a gas chromatograph for analysis of a-farnesene from volatile compounds (Solis-Solis et al., 2007).

The amount of α -farnesene was determined with a GC–MS (Shimadzu QP2010 GC–MS) fitted with a DB-WAX column (30 m × 2 mm internal diameter, 0.25 µm thickness; J & W, US) and a FID detector with helium carrier gas at 3 mL/min. The GC–MS was prepared at 250 °C for 2 h. Firstly, the column temperature was set at 40 °C for 2 min. Then it was increased to 150 °C for 3 min. After the column reached 220 °C by 10°C/min, it was finally programmed at 250 °C by 5 °C/min and the ionization energy of the mass spectrometer was programmed for 70 eV. Moreover, the ion source temperature was set at 250 °C and scanned at 25–425 m/e for 1 s. The WILEY and NIST libraries were used for identification of compounds.

2.4. Incidence and severity of superficial scald

The stored fruits were assessed for the presence of superficial scald at the end of 2, 4, and 6 months of storage. The incidence of superficial scald was expressed as a percentage of the affected fruits from the number of total fruits per replicate. On fruit showing scald symptoms, scald severity was rated on a scale of 0–4 based on the percentage of surface area affected, where 0 is no scald,

1 is 1%-10% (very low), 2 is 11%-33% (medium), 3 is 34%-66% (high), and 4 is 67%-100% (very high) (Zanella, 2003).

2.5. Fruit ethylene production

At the end of each of the storage periods, approximately 1 kg of fruit was kept at shelf-life conditions for 7 days and then put in air-proof plastic boxes for 24 h. At the end of this time, the data obtained from the ICA 56 ethylene analyzer were recorded as $\mu L/kg/h$.

2.6. Statistics

The experiment was established with a randomized complete plot design. There were three replicates for each treatment and 15 fruits per replicate. Data were subjected to analysis of variance (ANOVA) using Minitab 16. Means were separated using a least significant difference (LSD) multiple range test at P < 0.05.

3. Results and discussion

3.1. a-Farnesene concentration

Table 1 shows the α -farnesene percentages of the Granny Smith apples to which 1-MCP was applied at different concentrations under various storage conditions. The GC–MS analysis showed that the harvested Granny Smiths apples contained 2.55% α -farnesene out of the total volatile compounds.

 α -Farnesene percentages at the initial stage were lower than those of the 1-MCP-applied apples. α -Farnesene percentages obtained from the GC–MS analyses of the control fruits are shown in Figure 1. The α -farnesene percentage in the control group was 28.16% at the end of the second month of storage. This percentage dramatically decreased during storage. The reason for this decrease was auto-oxidation of α -farnesene under storage conditions. α -Farnesene auto-oxidation is associated with ethylene production, as demonstrated by Lurie and Watkins (2012).

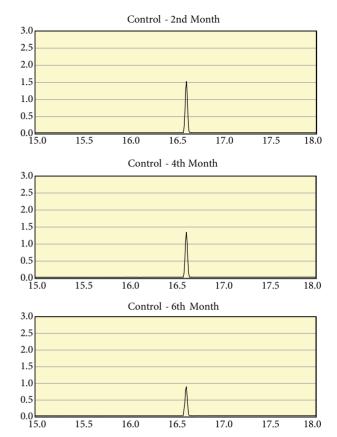


Figure 1. The change in α -farnesene in the control fruits during different storage durations.

According to the mechanism involved in the article 3*E*-conjugated-triene hydroperoxide oxidation could not be identified. However, a decrease in superficial scald formation showed that oxidation could be prevented by 1-MCP application.

Treatment	Initial (%)		Second month		Fourth month		Sixth month	
	α-farnesene concentration	Scald ratio (scald intensity)	α-farnesene concentration	Scald ratio (scald intensity)	α-farnesene concentration	Scald ratio (scald intensity)	α-farnesene concentration	Scald ratio (scald intensity)
Control (ppb)	2.55	-	28.16 b	2.2 (1)	26.88 d	50 (3)	16.76 c	84.4 (4)
312.5	2.55	-	65.70 a	-	78.11 a	6.7 (1)	11.33 d	10 (1)
625	2.55	-	12.38 c	-	60.85 b	2.2 (1)	30.96 b	4.4 (1)
1250	2.55	-	2.54 d	-	53.44 c	2.2 (1)	49.93 a	5.1 (1)
LSD	NS		3.2828		3.4563		3.7425	

Table 1. The effects of the 1-MCP applications on α -farnesene concentration and scald ratio (scald intensity) during storage (%).

Different letters refer to different statistical groups (P < 0.05), and NS = nonsignificant.

In our view, α -farnesene and 1-MCP can be easily oxidized similar to the Schenck ene reaction. This reaction describes how hydroperoxides formed by singlet oxygen in compounds containing the 1-methyl-ene unit could be converted to alcohols (Prein and Adam, 2003). This mechanism pathway is given in Figures 2a and 2b.

The 1-MCP applications clearly decreased α -farnesene oxidation, as indicated in Table 1 and Figure 2. The chromatographic investigation showed that there was still α -farnesene in the 1-MCP-treated apples at the end of 6 months of storage.

Furthermore, α -farnesene levels measured during storage of the control group remained lower. The control group contained 28.16% α -farnesene at the end of 2 months of storage, then 26.88% at the end of 4 months, and lastly 16.76% at the end of 6 months of storage.

The influence of 1-MCP treatment on α -farnesene content was dependent on the concentration of the application. The 1-MCP treatment of 312.5 ppb maintained higher levels of α -farnesene during the 2, 4, and 6 months of storage, at 65.70%, 78.11%, and 11.33%, respectively

(Table 1). Similarly, the 625 ppb 1-MCP treatment resulted in higher α -farnesene accumulation than the 312.5 ppb treatment at the end of 6 months of storage (30.96%). The highest α -farnesene percentages were determined in the 1250 ppb 1-MCP treatment at the end of 4 and 6 months of storage, as expected, at 53.44% and 49.93%, respectively.

α-Farnesene as an aromatic compound is primarily synthesized in apple skin (Kondo et al., 2005). The accumulation of α-farnesene increases during 8 to 12 weeks of cold storage, especially in scald-susceptible apple cultivars such as Granny Smith, Law Rome, and Delicious. These varieties typically exhibit a relatively high rate of α-farnesene synthesis shortly after they are placed in cold storage, which results in marked accumulation of sesquiterpene, including α-farnesene in the skin (Anet, 1972; Whitaker et al., 1997, 1998; Lurie and Watkins, 2012). The concentration of α-farnesene subsequently decreases and is converted to conjugated triene oxidation products during 16–24 weeks of cold storage, as pointed out by several authors (Huelin and Coggiola, 1970; Anet, 1972; Whitaker et al., 1997, 1998; Pechous et al., 2005).

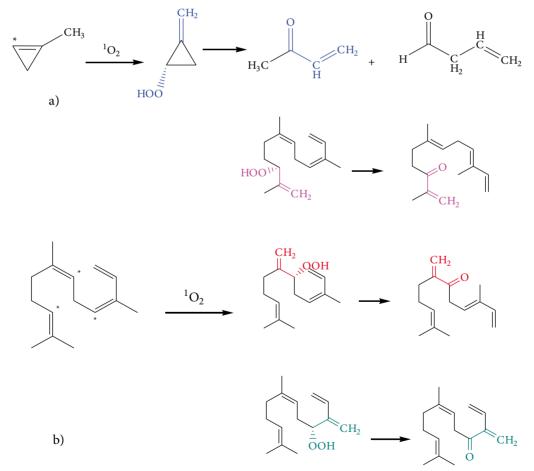


Figure 2. (a) The mechanism of the oxidation reaction of 1-MCP. (b) A schematic of the pathway of α -farnesene oxidation in apple fruit.

3.2. Scald percentages and intensity

Scald percentages and intensities of the stored Granny Smith apples are presented in Table 1. In this study, scald increased during storage for both the control group and the 1-MCP treatment groups. However, the 1-MCP treatments reduced the scald depending on the concentration of 1-MCP.

The first scald symptoms were observed in the second month of storage in the control group. Scald was at 2.2% at the end of 2 months of storage. After that, scald increased severely and reached 50% at the end of the fourth month and 84% at the end of 6 months of storage.

The 1-MCP treatments reduced the scald percentages from the lowest to the highest concentration. There was no scald development at the end of 2 months of storage for the 312.5 ppb treatment group. However, scald later reached 6.7% and 10% at this concentration. The 625 and 1250 ppb concentrations of 1-MCP both reduced scald. Both of these concentrations had a greater effect compared to 312.5 ppb for reducing scald development over a longer storage period. Scald was 2.2% for both 625 and 1250 ppb treatments after 4 months of storage. After 6 months of storage, the scald percentages were 4.4% for the 625 ppb treatment and 5.1% for the 1250 ppb treatment. Thus, 1-MCP treatment at 625 ppb prevented approximately 80% of scald development in Granny Smith apples after 6 months of storage (Figure 3). As previously reported, 1-MCP treatments reduced both a-farnesene oxidation and scald development (Fan et al., 1999; Watkins et al., 2000). Similarly, it has been reported that 1-MCP treatments reduced scald development by 30% in McIntosh and 90% in Delicious apple cultivars (Rupasinghe et al., 2000). DeEll et al. (2002) found that 1-MCP reduced scald development by 30% in Cortland apples. Our findings are in accordance with these studies.

3.3. Fruit ethylene production amount

Ethylene production rises during storage in climacteric fruits. In the present study, the ethylene amount changed

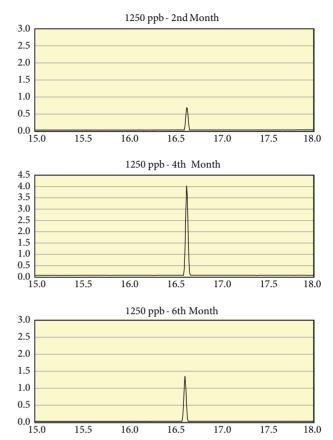


Figure 3. The effects of the 1250 ppb 1-MCP treatments on α -farnesene during different storage durations.

among the different applications and was found to be statistically significant (P < 0.05). The lowest ethylene concentrations were found for the 625 and 1250 ppb doses of 1-MCP (Table 2; Figure 4). Moreover, the effect of the applied doses differed significantly according to the duration of the storage period. The application of 1-MCP positively affected the delay of maturation. Defilippi et al. (2004) stated that 1-MCP treatments inhibit 70%

Table 2. The effects of various doses of 1-MCP treatments on the fruit ethylene production (μ L/kg/h) of Granny Smith apples during storage.

Treatment 1-MCP (ppb)	Storage duration				
	Initial	Second month	Fourth month	Sixth month	Average of treatment
Control	0.433 i	54.23 c	91.23 b	103.77 a	62.417 A
312.5	0.433 i	1.30 g	1.87 f	4.17 d	1.942 B
625	0.433 i	0.80 h	1.23 g	2.30 e	1.192 C
1250	0.433 i	0.77 h	1.27 g	2.27 e	1.183 C
LSD	0.08	3			0.083

LSD (0.05) Treatment × Duration: 0.167. Different letters refer to different statistical groups.

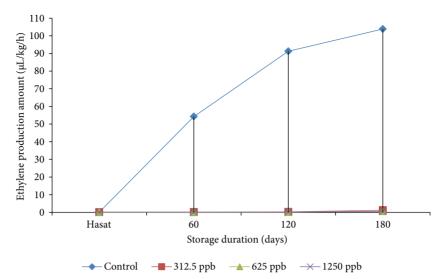


Figure 4. The effects of the 1-MCP treatments on ethylene production as μ L/kg/h during the different storage periods.

of ethylene production. 1-MCP applications retarded ethylene production in plum and apricot according to Dong et al. (2001). Similarly, ethylene production was almost stopped in Fuji (Fan and Mattheis, 1999), Red Delicious, and Granny Smith apples (Fan et al., 1998). Our results are in accordance with the previously published studies.

Superficial scald is an important physiological disorder of apples and pears. Scald can be prevented by decreasing α -farnesene production, α -farnesene oxidation, its chemical pathways, and fruit tissue resistance against α -farnesene related compounds (Watkins et al., 1995).

Ethylene promotes both maturation and α -farnesene production. Various researchers have stated that ethylene inhibitors may play an important role against scald development by decreasing the accumulation of α -farnesene and related compounds in Granny Smith and

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Red Delicious apples (Gong and Tian, 1998). In the present study, the application of 1-MCP had an important effect on controlling scald by decreasing ethylene production.

Natural antioxidants play an important role in preventing scald by delaying oxidation of α -farnesene (Anet, 1972). 1-MCP is a cyclic isoprene compound and an unstable molecule. It can react to reactive oxygen species (ROS). Therefore, 1-MCP can work as an antioxidant. In addition, there is no residue of 1-MCP in the fruits to which it is applied because of rapid gas formation at room temperature. 1-MCP has a positive effect against scald by preventing α -farnesene oxidation.

In summary, while the application of 1-MCP at 312.5, 625, and 1250 ppb doses all had a positive effect on scald inhibition in Granny Smith apples, the 625 ppb doses of 1-MCP in particular gave better results for decreasing scald by prevention of α -farnesene oxidation.

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