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Effect of UV-C radiation on postharvest physiology of persimmon fruit (*Diospyros kaki* Thunb.) cv. `Karaj´ during storage at cold temperature

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Abstract

Drastic softening and incidence of diseases are the limiting factors of persimmon postharvest life in Iran. Ultraviolet radiation at hormic doses has been used to control postharvest disease and delay softening in some fruits. Therefore, in this study UV-C radiation at the doses of 0 (as control), 1.5 and 3 kJm^{-2} were applied on the `Karaj´ persimmon and the quality attributes and disease incidence were evaluated after 0, 1, 2, 3 and 4 month storage at 1°C, plus 3 days shelf life. Results showed that both 1.5 and 3 $kJm^{-2}$ UV-C treatments compared to non treated fruits reduced the postharvest disease incidence of `Karaj´ persimmon during storage without any important effect on the fruit attributes such as, firmness, ethylene production and skin color. Hence, applied UV-C light after harvest could increase postharvest life of `Karaj´persimmon by disease control, but for having suitable firmness, it must be integrated with other effective postharvest treatments. However, it can be recommended for local markets, where the consumer prefers soft persimmon, same as Iran.

Introduction

In recent years persimmon fruit (*Diospyros kaki* Thunb.) production and consumption has increased noticeably in the world. The world-wide persimmon production was 2,417,602 tons in 2000. This increased to 3,263,021 tons in 2005 and more than 4,000,000 tons in 2010. China is the main producing country in the world. Persimmon production in Iran was 2100 tons in 2010, which represents 10th country in the world (FAO, 2010). But with increasing interest for developing the cultivation of persimmon, however there is not suitable technology and enough information about postharvest behavior and storability of this fruit in developing countries. As a result, persimmon producers cannot offer their production out of season and have obligate to sell it at low prices during a short period after ripening time. Our experience on the`Karaj´ persimmon in Iran showed that fast softening and the incidence of different diseases are limiting factors for postharvest life of this cultivar. In climacteric fruits, low temperatures can be used to achieve a delay in the onset of ripening and in result increase of postharvest life. On the `Karaj´ persimmon we indicated that fruits stored at 0°C had higher firmness and lower disease incidence than fruits stored at higher temperatures (Khademi *et al*., 2010). Effective postharvest treatments could become a possible alternative to cold storage for control disease incidence and maintaining fruit firmness. It must be noted that `Karaj´ persimmon is an astringent type but removing the astringent taste by artificial treatments is unusual in Iran and consumers prefer persimmon fruit at over ripe stage when astringent taste is lost naturally.

Since 1960s there had been doubts for control of fungi on fruits by synthetic anti-fungal compounds, because it had emerged that, a significant number of commonly used fungicides pose a threat to human health (Shama and Alderson, 2005). Consumers today are looking for fruit free of chemical residues, in result, alternative control strategies such as natural compounds, physical treatments and or use of antagonist organisms have been developed (Cia *et al*., 2007). Ultraviolet irradiation is widely used as an alternative strategy to control microorganism in food products (Stevens *et al*., 1996; Stevens *et al*., 1997; Nigro *et al*., 1998; Shama and Alderson, 2005; Jiang *et al*., 2010). UV light is a natural stress that can cause damage in plant tissues by inducing oxidative stress (Springob *et al*., 2003). Hormesis has been defined as tolerance stimulation by low doses of

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any potentially harmful agent (Shama and Alderson, 2005). Low doses of UV-C can inflict slight damage to plant tissue and activate repair mechanisms against this damage; and the repair mechanisms induce a positive reaction in the homeostasis function of organ (Luckey, 1980).

Reduction of decay under hormic doses of UV-C treatment is due to its germicidal effect on pathogens and or induction of resistance inside the plant tissue (Cia *et al*., 2007), but it seems that induced resistance is more important than germicidal effect. Chalutz *et al*. (1992) indicated that, on the grapefruit, the peel regions not directly exposed to UV-C also exhibited resistance to infection and they suggested that a resistance induction signal may be transferred within the fruit. Charles *et al*. (2008a, 2008b, 2008c, 2008d and 2009) in a comprehensive study showed that susceptibility of tomato fruit to *Botrytis cinerea* increased immediately after UV-C treatment, however, the resistance in the UV-C treated tomato increased gradually and reached to maximum at 12 days after treatment, and therefore the induced resistance remained effective throughout the storage period of 35 days. They confirmed that early resistance against infection is dependent upon phytoalexins accumulation in UV-C treated fruit, but prolonged resistance related to different mechanisms such as formation of cell wall stacking zone as a barrier against pathogen infection, trigger of phenylpropanoid pathway that leads to accumulation of single and complex phenolics, and enhanced synthesis of several constitutive and pathogenesis-related proteins.

Black spot caused by *Alternaria alternate* is an economically important postharvest disease of `Triumph´ persimmon fruit. Spores of *Alternaria alternate* landed on the fruit surface in the orchard remain quiescent, and when the fruit is harvested and thereafter begin to develop during postharvest life (Prusky *et al*., 2001; Prusky *et al*., 2006). Therefore, germicidal effect of UV-C radiation on fungal spores can not be effective to prevent persimmon infection during storage and induced resistance must be generated.

Jiang *et al*. (2010) showed that UV-C treatment maintained a high level of mushroom firmness during cold storage and the following shelf life at 20°C. The effect of UV-C treatment on reduction of the activity of cell wall degrading enzymes was confirmed by Pombo *et al*. (2009) on the strawberry. UV-C treatment by production of higher polyamines level, reduced ethylene production and action and decreased activity of cell wall-degrading enzymes, could delay fruit ripening and tended to increase postharvest life of horticultural products (Maharaj *et al*., 1999; Shama and Alderson, 2005; Costa *et al*., 2006). There is not any report on the effect of UV-C treatment on persimmon fruit; therefore the objective of this study was to evaluate the response of 'Karaj' persimmon to UV-C radiation after harvest.

Materials and Methods

Materials

The persimmon fruit cv. `Karaj´ was harvested at mid-November of 2009 from a commercial orchard near to Karaj city (35 km west of Tehran, Iran) and immediately was transported to the Department of Horticulture Science, University of Tehran. Fruit at harvest presented L* value of 57, hue vale of 67.5 and firmness value of 54N. Fruits without wounds or rots were sorted for uniform size and maturity.

Methods

UV-C treatment

For treatment, a germicidal low pressure mercury vapor discharge lamp (Philips, Holland) having nominal power output of 30W, emitting quasi-mono chromatic UV radiation energy at 254 nm, was used. Three lamps were mounted over the radiation vessel and fruits were placed below the lamps at a distance of 30 cm. The influence rate of the lamps at the level of samples was measured with a digital UV-meter (LT Lutron; D.56013, Taiwan). The average dose rate was 0.63 mW cm^{-2}. The UV-C radiation doses adjusted by altering the duration of the exposure time at the fixed distance.

Maximum UV dose was determined ultimately by the appearance of undesirable changes on the fruit; these changes include skin discoloration, browning and fruit drying (Shama and Alderson, 2005). To select the most suitable experimental conditions at a preliminary trial, top and bottom parts of `Karaj´ persimmon fruits were irradiated with different doses of 1, 1.5, 3, 4, 5, 7 and 10 kJm^{-2} of UV-C light and then stored at 20°C for 4 day in darkness for acceleration of skin damage. After the preliminary experiment, the doses of 1.5 and 3 kJm^{-2} were selected as suitable and desirable UV-C doses and were applied on the fruits as UV-C treatments. After the treatments, fruits stored at 1°C and humidity of >80% at darkness. To evaluate the effect of UV-C treatments on disease incidence and fruit properties, from Mid-November of 2009 until Mid-March of 2010, 15 fruit of each group were sampled on months of 0, 1, 2, 3 and 4 and then were maintained for 3 days at room temperature as shelf life. Then the fruit properties were measured.

In a separate experiment, to determine the effect of UV-C light on ethylene production rate of `Karaj´persimmon, 45 fruits were treated with selected doses (0, 1.5 and 3 kJm^{-2}) of UV-C and after 12, 24 and 72 hours the ethylene production rate were measured by 5 fruit of each treatment.

Fruit assessment

Persimmon fruit samples were weighed before and after storage to calculate percentage of weight loss during storage. Superficial color was determined by measuring parameters of L*, a* and b* with a chroma meter (Minolta CR-400, Japan) at three points of fruits. The hue angle was calculated by formula of $h° = tan^{-1}$ (b*/a*). Fruit firmness (N) was determined by using of hand penetrometer (FT 327, Italy) equipped with 8 mm cylindrical head. Total Soluble Solid (TSS) was determined by a hand refractometer (RF40) using fruit juice. Titratable acidity (TA) was determined by titration of fruit juice with 0.1 mol/L NaOH up to pH 8.1, the results were expressed as gram of malic acid per 100 gr fresh weight. The soluble tannin content was determined by Folin-Denis method (Taira, 1996). To determine ethylene production rate, the fruits were placed within 2000 ml plastic jars at 25°C for 1 hour, then headspace gas samples (1ml) were withdrawn from the jars by a syringe and injected into a gas chromatograph (Model 14A, Shimadzu, Japan) equipped with a flammable ionization detector. Persimmon fruit were regarded as unmarketable when more than 1% of the fruit surface area exhibited fungal decay (Prusky *et al*., 2006), therefore the fungal decay incidence were evaluated according to the number of the fruits from each treatment, those deteriorated by fungi, when the fruit transferred from storage to shelf life and the percentage of fruit decay incidence calculated as: (number of fruit with incidence /total number of fruit in treatment) ×100 according to Özdemir *et al*. (2009).

Statistical analysis

A number of 225 fruits were randomly assigned at three groups, each group included 75 fruits. One group was treated with 3 kJm^{-2} of UV (at duration of 8 min) and the other treated with 1.5 kJm^{-2} UV (at duration of 4 min) radiation and other untreated group considered as control. The experiment was a 3×5 factorial design (UV-C treatment ×storage time) which was subjected to analysis of variance (ANOVA) using SAS (ver. 9.1) software. Mean values were subjected to the least significant difference test (LSD) at $P<0.05$. Figures represent mean values (n=3) ± SE.

Results and Discussion

Skin color properties

Three days after UV-C light treatments the Luminosity (L*) of fruits decreased slightly compared to control, but during 4 month of storage at 1°C, in spite of decrease in skin color Luminosity by time, no significant difference was observed between UV-C treatments and control (Figure 1). The slight significant decrease of L* immediately after the UV treatment probably related to initiation of slight browning in fruit surface that stopped during storage at low temperature. On the tomato fruit, Charles *et al*. (2008c) indicated that UV-C treatment altered the amount of epicuticular wax and its ultra structural properties, by change in its chemical compositions. These changes could affect light reflectance characteristics of the fruit surface. Decrease in L* during storage indicated that fruits become darker by progress in coloring (Cia *et al*., 2007). Vicente *et al*. (2005) showed that UV-C treatment did not affect the L* of the pepper fruits after storage.

The hue angle value also decreased in all samples during storage and no significant difference was observed between UV-C treatments and control according to hue angle (Figure 2). Decrease in hue angle is characteristic of advanced stage of ripening and change of color from yellow–orange to red–orange that displayed by `Karaj´ persimmon when it's ripening took place.

Ethylene production

Study of the pattern of ethylene production after the exposure to UV-C light showed that, 12 hours after the treatment there was no significant difference among the samples according to ethylene production, but after 24 hours, the ethylene production rate of 3 kJm^{-2} UV-C treated fruits was significantly higher than that of control and 1.5 kJm^{-2} UV-C treatment. However, 48 and 72 hours after the treatment no significant difference was observed in ethylene production rate among the applied treatments (Figure 3a). Also during storage, the ethylene production rate increased by first month and then remained constant or decreased slightly until the end of storage. However, there was no significant difference among the samples according to ethylene production rate during storage (Figure 3b). These results indicated that UV-C treatment at its higher doses, same as other stress, can stimulate ethylene production in persimmon fruit (Nakano *et al*., 2001), but in spite of its primary effect, 3kJm^{-2} UV-C treatment could not change the ethylene production rate during storage; therefore its damage has been repaired by cells

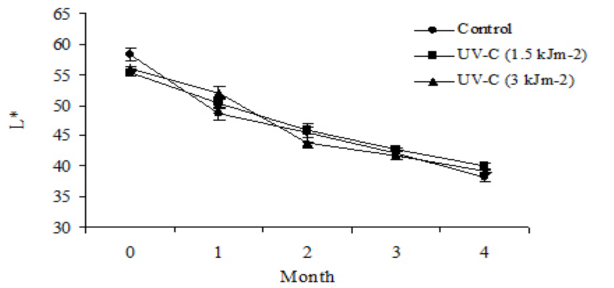

Figure 1. Changes in Luminosity (L*) of persimmon fruit cv. `Karaj´ during cold storage (+1°C) plus 2 days shelf-life at 25°C after UV-C treatments at harvest. Vertical bars indicate standard error (SE) of means

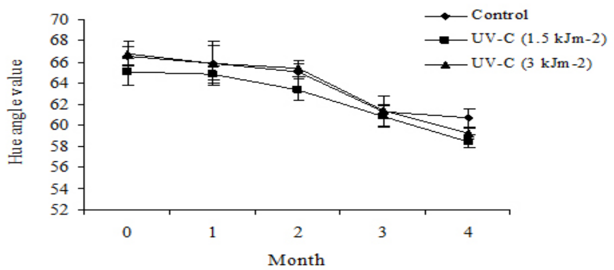

Figure 2. Changes in hue angle value of persimmon fruit cv. `Karaj´ during cold storage (+1°C) plus 2 days shelf-life at 25°C after UV-C treatments at harvest. Vertical bars indicate standard error (SE) of means

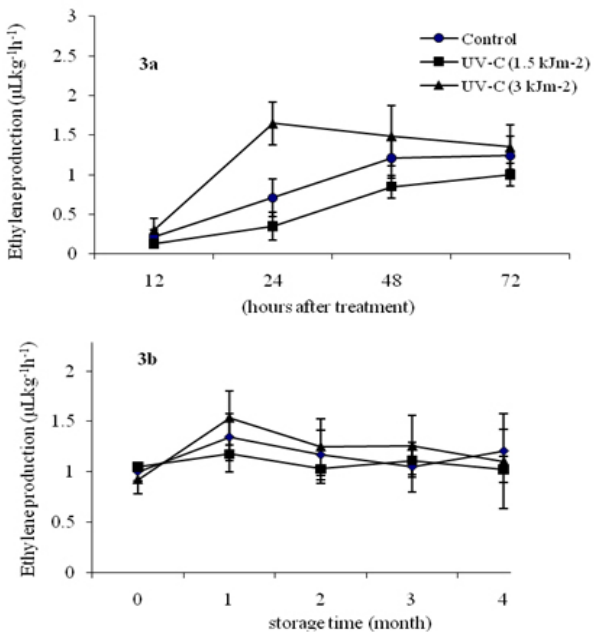

Figure 3. Change in ethylene production rate ($\mu Lkg^{-1}h^{-1}$) of persimmon fruit cv. `Karaj´ after UV-C treatments at harvest (3a), and during cold storage (+1°C) plus 2 days shelf-life at 25°C (3b). Vertical bars indicate SE of means

during cold storage. In fact, UV-C light produces stress inside the plant by oxidative reactions and plant responds to this stress by inducing scavenging enzymes and key enzymes related to synthesis of a number of antioxidant compounds. At sub-lethal doses, the plant repairs UV-C damage and the positive changes were conferring in cells protect them against incoming stress, a phenomenon that named hormesis (Springob *et al*., 2003; Shama and Alderson, 2005).

Fruit firmness

A decrease in fruit firmness was observed in treated and non-treated fruits during storage, without differences among the treatments. This decrease was drastically during first and second months of storage and then became constant (Table 1). This result is in contrast with those reported for tomato fruit (Barka *et al*., 2000), pepper (Vicente *et al*., 2005), strawberry (Pombo *et al*., 2009) and shiitake mushrooms (Jiang *et al*., 2010). For these products, it was shown that UV-C irradiation maintained the firmness higher than control by reduction in activities of cell wall degrading enzymes. Persimmon fruit is very sensitive to ethylene and exposure to low amount of ethylene accelerates its ripening (Salvador *et al*., 2004). Nakano *et al*. (2001) showed that persimmon softening was controlled by internal ethylene production. In present results, increase in ethylene production at early month of storage coincided with sharp fruit softening at the same time. Firmness is one of the most important quality attributes to be considered in the commercialization of persimmons and fruits with firmness value below 10N are unmarketable (Salvador *et al*., 2004). At this study firmness of `Karaj´ persimmon fall down less than 10N after two months of cold storage, and it seems that, the effective storage life of this cultivar was not more than two months. However, it has been confirmed that Iranian consumers preferred soft persimmon fruit at gelling stage that loose its astringent taste naturally (Mostofi *et al*., 2008).

Quality attributes

The TSS of fruit gradually increased and the TA slightly decreased during the storage. The differences among the treatments in TSS and TA were not significant (Table 1). Similarly, Perkins-Veazie *et al*. (2008), on the blueberry, showed that the TSS and TA were not affected by UV-C treatment. Also, Vicente *et al*. (2005) indicated that when sugar content and pH of pepper fruit juice were analyzed, no significant differences were found between non treated and UV-C treated fruit.

Results also showed that the soluble tannin content of fruit was affected by storage time but not with UV-C light treatments (Table 1). The soluble tannin content decreased markedly during storage without any significant differences among the applied treatments. Reduction of soluble tannin content during storage related to the complex formation between the

Table 1. Changes on fruit firmness, total soluble solid (TSS), titratable acidity (TA%), soluble tannin content and weight loss of persimmon fruit cv. `Karaj´ during cold storage plus subsequent 2 days shelf life at 25°C after UV-C treatments on harvest

Months	UV-C doses	Firmness (N)	TSS (%)	Soluble tannin (ppm)	TA (%)	Weight loss (%)
0	**0 kJ/m²**	50.11	15.67	6984	0.156	0.75
	1.5 kJ/m²	48.41	13.33	6904	0.165	1.62
	3 kJ/m²	47.16	15.50	6937	0.151	1.88
1	**0 kJ/m²**	20.83	16.83	4989	0.151	2.29
	1.5 kJ/m²	18.58	16.67	5784	0.142	2.65
	3 kJ/m²	18.66	18.23	5911	0.142	2.85
2	**0 kJ/m²**	10.11	17.00	2523	0.136	4.44
	1.5 kJ/m²	12.66	18.00	2953	0.138	4.87
	3 kJ/m²	9.40	19.40	2658	0.118	6.9
3	**0 kJ/m²**	5.93	17.17	1994	0.136	8
	1.5 kJ/m²	6.58	18.17	2510	0.129	6.35
	3 kJ/m²	6.57	19.07	1933	0.111	8.5
4	**0 kJ/m²**	7.52	19.00	2297	0.132	9.13
	1.5 kJ/m²	6.96	18.37	1982	0.125	10
	3 kJ/m²	5.21	18.73	2210	0.11	10.2
LSD ($P \leq 0.05$)=		5.1	2.66	1249	0.031	4.44

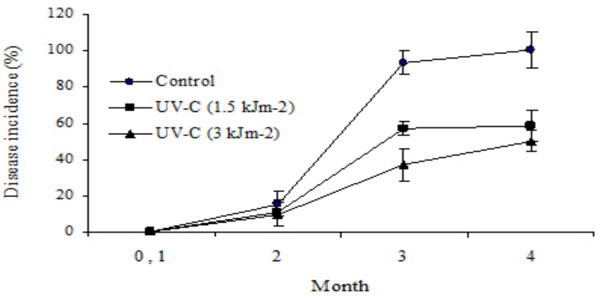

Figure 4. Changes in disease incidence (%) of persimmon fruit cv. `Karaj´ during cold storage (+1°C) plus 2 days shelf-life at 25°C after UV-C treatments at harvest. Vertical bars indicate SE of means

pectin released from cell wall, and tannins (Taira *et al*., 1997). Perkins-Veazie *et al*. (2008) on the blueberry, and Jiang *et al*. (2010) on the mushroom, showed that UV-C light treatment did not affect the amount of total phenolic content. The weight loss of fruits increased significantly during storage up to about 10% but was not affected by UV-C treatments (Table 1).

Disease incidence

Until two months after the treatments no any disease symptom was observed on the fruits. But thereafter the disease incidence began to develop in all samples and reached to maximum level in control fruits at the end of storage. Results indicated that the applied UV-C treatments induced resistance to disease incidence compared to control during storage, among these, the 3 kJm^{-2} UV-C light conferred more resistance than 1.5 kJm^{-2} UV-C light, however at the end of the storage no significant difference was observed between two UV-C treatments in disease incidence (Figure 4). This result is in accordance with reports showing that low doses of UV-C reduced the development of green mold rot on grapefruits (Chalutz *et al*., 1992), brown rot on peaches, *Alternaria* rot and bitter rot on apples, stem end rot and sour rot on tangerine (Stevens *et al*., 1996), *Rhizopus* soft rot on tomato and sweet potato (Stevens *et al*., 1997), *Botrytis cinera* on grape berries (Nigro *et al*., 1998), *Botrytis* as well as *Alternaria* on pepper (Vicente *et al*., 2005), Fusarium on the gladiolus corms (Sharma and Tripathi, 2008), *Botrytis cinera* on tomato (Charles *et al*., 2008d) and mesophilic, psycrophilic and entero bacteria population on fresh-cut watermelon (Artes-Hernandez *et al*., 2010). In contrast, Lopez-Rubira *et al*. (2005) on the pomegranate arils and Cia *et al*. (2007) on the papaya showed that UV-C irradiation was not able to reduce the incidence of relative pathogens.

Primary resistance during early month is contributed to high level of soluble tannins and firm texture of fruit. But, after the soluble tannin reduction and fruit softening, resistance of irradiated fruits compared to control could be contributed to induce resistance inside the fruits by UV-C light. UV-C light results to the synthesis of a number of anti-fungal compounds in the plant organs such as; phytoalexins, pathogensis-related proteins (chitinases, glucanase), flavonoides, phenolic acids, lignin, suberin, and induces the activity of anti-oxidant enzymes such as catalase, peroxidase, ascorbate peroxidase and PAL. These components and enzymes restrict the fungal growth on the fruit and decrease disease incidence severity (Shama and Alderson, 2005; Charles *et al*., 2008b; Charles *et al*., 2008d; Charles *et al*., 2009; Wang *et al*., 2009; Jiang *et al*., 2010). No noticeable browning symptoms were observed in all samples at this experiment (data not shown).

Conclusion

The result showed that, both 1.5 and 3 kJm^{-2} UV-C light treatments could increase postharvest life of persimmon fruit cv. `Karaj´ by inducing resistance to different diseases without noticeable effect on the fruit quality attributes such as titratable acidity, total soluble solids and soluble tannin content. UV-C treatment did not affect ethylene production and fruit firmness of persimmon fruits during storage and hence the effects of this treatment in disease control are due to its direct effect on fruit physiology or fungal spores. It seems that 1.5 kJm^{-2} UV-C treatment is better than 3 kJm^{-2} UV-C due to less stress to fruits. According to the importance of firm fruits for transportation and marketing, however, UV-C light in this study could not maintain it. As a result, for use of positive effect of UV-C light treatment on disease control as well as having firm persimmon fruits, it must be integrated with other effective postharvest treatments. However Iranian consumers prefer gel-like persimmon, which is very prone to disease, and therefore UV-C treatment in Iran can be commercially used for persimmon postharvest only for disease reduction.

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