Heat Conditioning before Ultraviolet-C Illumination Improves Decay Control and the keeping Quality of Cold Stored Lemon Fruit

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Abstract

In order to avoid rind damage following postharvest UV-C light treatment on sensible *Citrus* cultivars the illumination was performed after heat conditioning (HT). Experiments were performed with lemons (*Citrus limon* 'di Massa') harvested twice (April and June), graded and divided into 6 sets (each of 180 fruit) according to HT duration (hours) as follows: I) none; II) 3 h; III) 6 h; IV) 12 h; V) 24 h; and VI) 36 h. HT was performed in a ventilated room at 36 °C under saturated RH conditions. After HT, half of the fruit (90 lemons) of each set was treated with 6 kJm^{-2} and half remained un-illuminated. Then, all fruit was stored for 2 months at 5 °C and 90±5% RH, followed by a 6 day simulated marketing period (SMP) at 20 °C and 75% RH. After 1 month, at the end of storage and SMP rind damage was scored and the percentage of decay monitored. Rind disorders caused by UV-C illumination varied upon harvest time and HT duration. In the combined treatments the restrain of UV-C induced damage was achieved from 12 h HT on, and among 12, 24 and 36 h of HT differences were not significant. Fruit subjected to 24 or 36 h HT were free of rind disorders at the end of the experiment. During storage rots were mainly caused by *Penicillium italicum* while *P. digitatum* was the main cause of decay during the SMP. The combined treatments had synergistic interaction in controlling the decay when HT was performed for 12, 24 or 36 h. The greatest weight loss after 1 month of storage was found in fruit subjected to 24 and 36 h of HT followed by the UV-C illumination while, after 2 months and following SMP differences among treatments were negligible.

INTRODUCTION

The demand for alternatives to synthetic postharvest fungicides has implemented the researches on biological control agents, compounds generally recognized as safe (GRAS), physical methods along with studies focused at enhancing fruit natural resistance. In this direction, the induction of natural resistance, following biotic or abiotic stresses is gaining interest and postharvest implementation is being considered (Ben-Yehoshua and Mercier, 2005). Postharvest treatments with ultraviolet-C (UV-C, 254nm) light are among these technologies and have been positively correlated to the reduction of decay during cold storage of *Citrus* fruits. Hormesis effects of UV-C illumination have been reported on a large number of fruits and vegetables (Charles and Arul, 2007). The efficacy of UV-C illumination in controlling decay is dose dependent and it was also evidenced to be strictly related upon cultivar and harvesting time (D'hallewin et al., 1997). In addition, the defence against infection was achieved when illumination was applied before inoculation with *Penicillium digitatum* Sacc., without direct exposure of he pathogen to UV-C light. Moreover, when inoculation was performed 24 h before illumination the treatment failed to prevent decay development. These experiments evidenced clearly that the mode of action, in preventing decay, was associated to the build up of natural resistance. When Kumquat (*Fortunella Margarita*) fruit was artificially inoculated after illumination, the decay development was inhibited from the 5th day post-treatment and was lost 15 days later. Quantization of the two main induced phytoalexins, evidenced an accumulation pattern with a peak between 9 to 11 days post-illumination and a complete loss within 18 - 20 days. This pattern together with the induction of chitinase and β-1,3 endoglucanase (Porat et al. 1999) can explain the results of the artificial inoculation experiment with Kumquat fruit and support the role of the induced natural resistance. In addition, scoparone (6,7 dimethoxy coumarin), one of the induced phytoalexins by UV-C light in the flavedo of *Citrus* fruits is well known and largely used in Asian traditional medicine. The health promoting property of this compound adds a considerable functional value to those fruits where the rind is edible (e.g. Kumquats). Still, under particular circumstances rind damages have been reported with a considerable loss of market value. To avoid rind staining and pitting of sensitive *Citrus* fruits attempts have been made by combining UV-C illumination with heat treatments (D'hallewin et al. 1994; Ben-Yehoshua et al. 2005). Heat transfer to commodities can be performed either by water or hot air and both methods contain postharvest decay and prevent rind staining and pitting during short storages. The results reported on the combined heat/ UV-C treatments have evidenced complex interactions between the two treatments depending upon the mode of heat transfer and the sequence of application (Ben-Yehoshua et al. 2005). The most promising results were achieved by curing (heat transfer by heat-humid saturated air) the fruit before UV-C illumination. This sequence enhanced the accumulation of phytoalexins, controlled 24 h infections, prevented rind damage and improved the fruit keeping quality during long storage. Despite these good results, the duration of the treatment to cure *Citrus* fruits was too long (72 h) from a practical point of view. Thus, we considered finding a combination with a shorter curing duration and a synergic effect on decay control. Here we report the results obtained with lemon fruit cured for 3, 6, 12, 24 or 36 h before illumination with 6 kJm^{-2}.

MATERIALS AND METHODS

Fruit. Lemon fruit (*Citrus limon* 'di Massa') was harvested twice (April and June) and after grading divided into 6 sets (each of 180 fruit), according to the curing (HT) length.

Curing and UV-C Illumination. The heat treatment was performed by keeping lemons in boxes (60 fruit each) in a ventilated room at 36 °C with a saturated relative humidity (RH). According to the HT duration (hours) the sets of lemons were treated as follows: I) none; II) 3 h; III) 6 h; IV) 12 h; V) 24 h; VI) 36 h. Following HT, within each group, half of the fruit (90 lemons) was treated with 6 kJm^{-2} and half remained unilluminated. The light treatment was performed according to D'hallewin et al**.** (1999) **.** Then, all fruit was moved to cold storage in the dark.

Storage and Simulated Marketing Period. Lemons were stored for 2 months at 5 °C and 90±5% RH. After storage, all fruit was moved to a ventilated room at 20 °C and 75% RH for additional 6 days, simulating a marketing period (SMP).

Rind Damage and Decay. After 1 month, at the end of storage and SMP, the degree of rind damage was rated and a damage index calculated according to Lafuente et al. **(**1997**)**. At the same time, the amount of fruit affected by moulds was monitored and expressed as total percentage of decay.

Weight Loss and Total Visual Assessment. Fruit weight loss was monitored during storage and SMP by weighting each fruit every two weeks and at the same time also the visual assessment was performed based on a 1 to 3 scale, where 1, 2 and 3 represented excellent, good and poor appearance, respectively.

Statistical Analysis. All data were subjected to ANOVA using the statistical program OpenStat (2007) and where appropriate mean separation was performed according to the Newman-Keuls Test. Decay percentage was transformed to Bliss angular values before ANOVA and actual means are reported in figures.

RESULTS

Rind damage. Rind damage caused by the UV-C illumination was strictly depended upon harvest time. Fruit from April was more sensible and the damage index value after SMP was 2.2 (Fig. 1). The harvest in August provided fruit less sensitive to UV-C damage and the index value of illuminated fruit was half that of April at the end of the experiment (Fig. 2). In the combined treatments, as duration of HT was increased from 3 to 12 h, a significant reduction of rind damage occurred on fruit of both harvests, while among 12, 24 and 36 h of HT, differences were negligible. When fruit was harvested in April, 3 and 6 h of HT were not effective in preventing rind damage while when harvested in August a significant, but not satisfactory, reduction took place after 6 h of HT. Fruit from both harvest, subjected to 12, 24 and 36 h of HT was free of rind disorders during the whole experiment.

Decay. Moulds, mainly caused by *Penicillium italicum* during storage, and *P. digitatum* during SMP were significantly reduced by combining the treatments, and the results were synergistic when HT was performed for 12, 24 and 36 h (Fig 3, 4). The control of decay during storage of fruit harvested in April was satisfactory with all treatments while, after the SMP only 12, 24 and 36 h of HT alone or combined with UV-C assured an effective control of decay (Fig. 3). In control fruit the natural decay during storage was lesser when harvested in August compared to April. Again, in fruit harvested in August, the best results were obtained by combining the two treatments and after SMP the decay was the lowest with 24 h HT combined with UV-C illumination. Synergic interactions were found only during storage with 12, 24 and 36 h HT combined with UV-C while this effect was attained only with 24 h HT during the SMP.

Weight loss. Weight loss was greater in early harvested lemons but the pattern between the two harvests was the same and the loss took place mostly during the first month of storage, while, at the end of storage and SMP no differences were found (Table 1). During the first month of storage the loss was greater in fruit subjected to HT for 24 and 36 h followed by UV-C illumination. During storage fruit illuminated with UV-C light had a greater weight loss compared to control fruit and when combined with 3, 6 and 12 h of HT it was reduced whereas after the SMP no differences among treated and untreated fruit was found.

Visual Assessment.

Fruit treated with UV-C light, besides the appearance of rind damage, remained green during the whole experiment while, those subjected to HT proceeded from green to yellow during the first month of storage. When the two treatments were combined the effect of UV-C light was lost when HT was performed for 24 and 36 h. When at harvest, fruit was partially green and yellow, the visual assessment was negatively influenced because at the end of SMP, the rind of UV-C illuminated or 3, 6, and 12 h HT illuminated fruit resulted yellow with dark-green spots and was scored poor. When fruit rind was predominantly green or yellow this effect was less evident and fruit was scored good. Fruit cured for 24 or 36 h before illumination turned yellow and were also scored good at the end of SMP. Harvest time and HT duration influenced the visual assessment when UV-C illumination induced rind damage.

DISCUSSION AND CONCLUSIONS

The two treatments alone improved the control of decay and HT additionally prevented the loss of quality. UV-C illumination resulted effective in keeping the fruit green which was good when the rind of lemon fruit was green. The combined treatment led to synergic effects when HT was performed at least for 12 h. Shorter HT did not prevent the appearance of UV-C light damage. It has to be pointed out that 6 kJm^{-2} is a rather high dose, since normally, when the treatment is applied alone, doses between 1.5 and 3 kJm^{-2} are used. Here we used a higher dose in order to induce damage and to be sure about the curative effect of the heat treatment. The beneficial effect of the HT was evident in early harvested lemons when it was performed for 12, 24 or 36 h, while, for late harvested fruit only 24 and 36 h were effective. Considering the whole experiment we can conclude that using a high dose 24 h of HT is the best compromise to avoid damage and control effectively pathogen development. Since pathogen development was greatly affected by the combined treatment probably at lower doses 12 h of curing, may not be adequate to obtain good results. Based on these results we plan to study the physiological bases of these results.

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Figures

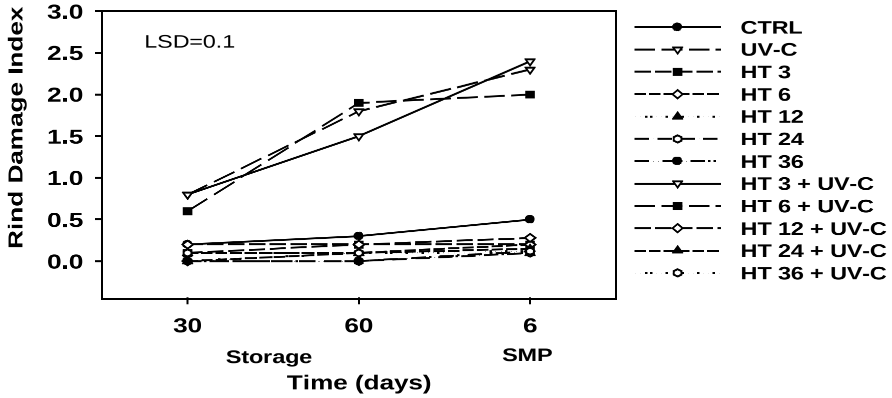

Fig. 1 – Index of rind damage (0 = none; 1 = slight; 2 = intermediate; 3 = heavy) on 'Di Massa' lemons harvested in April and kept in humid saturated air at 36 °C (HT) for 3, 6, 12, 24 or 36 h and then illumination with 6 kJm^{-2} of UV-C light (UV-C); un-treated fruit was used as control (CTRL). Fruit was stored at 5 °C at 95% RH and during the simulated marketing period (SMP) the temperature was 20 °C and the RH was 75%.

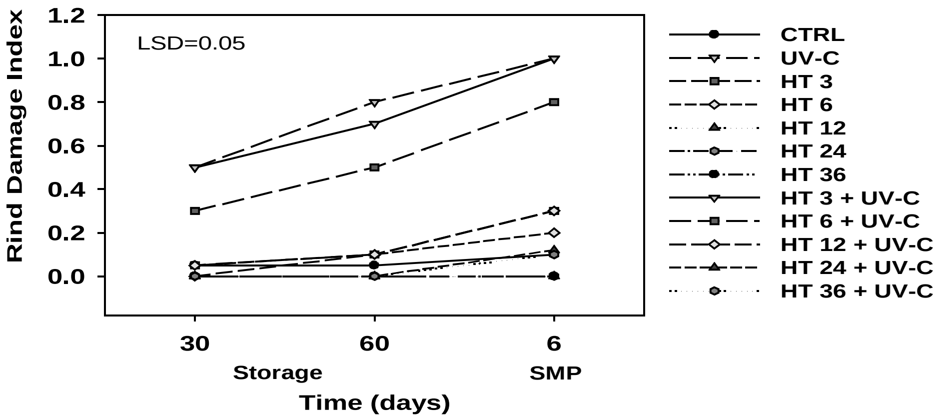

Fig. 2 – Index of rind damage (0 = none; 1 = slight; 2 = intermediate; 3 = heavy) on 'Di Massa' lemons harvested in August and kept in humid saturated air at 36 °C (HT) for 3, 6, 12, 24 or 36 h and then illumination with 6 kJm^{-2} of UV-C light (UV-C); un-treated fruit was used as control (CTRL). Fruit was stored at 5 °C at 95% RH and during the simulated marketing period (SMP) the temperature was 20 °C and the RH was 75%.

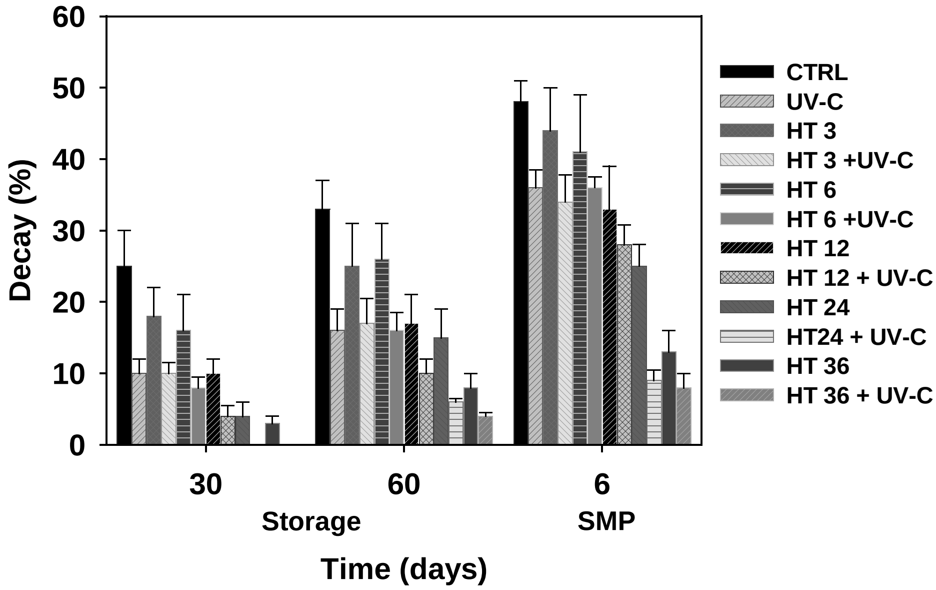

Fig. 3 – Decay percentage on ‘Di Massa’ lemons harvested in April and kept in humid saturated air at 36 °C (HT) for 3, 6, 12, 24 or 36 h and then illumination with 6 kJm^{-2} of UV-C light (UV-C); un-treated fruit was used as control (CTRL). Fruit was stored at 5 °C at 95% RH and during the simulated marketing period (SMP) the temperature was 20 °C and the RH was 75%, bars indicate ± SE.

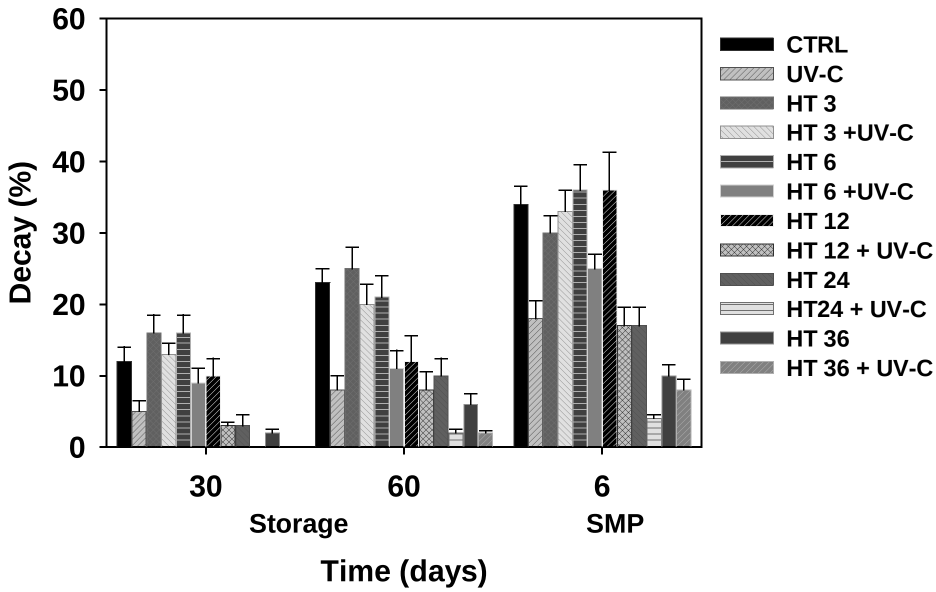

Fig. 4 – Decay percentage on ‘Di Massa’ lemons harvested in August and kept in humid saturated air at 36 °C (HT) for 3, 6, 12, 24 or 36 h and then illumination with 6 kJm^{-2} of UV-C light (UV-C); un-treated fruit was used as control (CTRL). Fruit was stored at 5 °C at 95% RH and during the simulated marketing period (SMP) the temperature was 20 °C and the RH was 75%, bars indicate ± SE.