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UV-C irradiation reduces microbial populations and deterioration in *Cucurbita pepo* fruit tissue

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Abstract

Tissue slices of zucchini squash (*Cucurbita pepo* L., cv. Tigress) fruit were exposed to ultraviolet-C (UV-C) radiation from germicidal lamps for 1, 10 or 20 min; however, only 10 and 20 min UV-C exposure significantly reduced microbial activity and deterioration during subsequent storage at 5 or 10°C. UV-C treated slices had higher respiration rates than controls; however, the ethylene production of the slices was not affected by UV-C treatments. Slight UV-C irradiation damage (reddish brown discoloration) was detected on the surface of 10 and 20 min-treated slices after 12 days of storage at 10°C. Slices stored at 5°C did not show UV-C damage. Chilling injury was not observed until after 20 days of storage at 5°C. The symptoms of chilling injury appeared as dried sunken brown spots on the surface of cortex tissue. UV-C treatments did not affect the degree of chilling injury during storage at 5°C. UV-C treatment also had no consistent effect on sugar or malic acid concentrations. The most pronounced effect of UV-C irradiation was to retard microbial growth thereby providing a basis for the frequently observed delay in senescence and subsequent deterioration in fruit tissues. © 2001 Elsevier Science B.V.

Keywords: Chilling injury; *Cucurbita pepo*; Ethylene; Respiration; UV-C irradiation

1. Introduction

Numerous studies have demonstrated the effectiveness of UV-C (254 nm) radiation from germicidal lamps in reducing deterioration of fruits and vegetables. Exposure to low UV-C doses has been reported to reduce postharvest decay of onions (*Allium cepa* L.) (Lu et al., 1987), sweet potatoes (*Ipomea batatas* L.) (Stevens et al., 1990), carrots (*Daucus carota* L.) (Mercier and Arul, 1993), and tomatoes (*Lycopersicon esculentum* Mill.) (Liu et al., 1993; Maharaj, 1995). However, the basis for this protection and the nature of plant-microbe interaction has not been clearly established.

It is well known that plant tissues produce increased amount of ethylene when subjected to

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various biotic and abiotic stresses (Mattoo and Suttle, 1991). The stimulation of ethylene production is an early biochemical event in many plant-pathogen interactions (Boller, 1991). Exposure to UV-C irradiation has been shown to induce resistance against pathogens in a number of species (Stevens et al., 1996; Nigro et al., 1998). The increased resistance may be related to the biosynthesis of substances toxic to the pathogens, induced by an increase in the activity of enzymes such as phenylalanine ammonia-lyase (Frietzenheimer and Kindl, 1981). The levels of antioxidants (α-tocopherol, β-carotene, and ascorbic acid) were increased by UV treatment in several green vegetables (Higashio et al., 1999). However, the effect of UV-C irradiation on changes in microbial population and the in vivo production of ethylene and senescence in *Cucurbito pepo* has not been determined. UV-C effects on soluble sugar and organic acid contents and respiration rate are also not known. The primary aim of this study was to test the hypothesis that the protective effect of UV-C irradiation is related to the reduction in microbial population on the surface of fruit tissues and to determine the relationship between microbial activity and production of ethylene and other constituents.

2. Materials and methods

2.1. Experimental treatment

Zucchini squash (*Cucurbita pepo* L., cv. Tigress) were harvested in October 1998 from one of the commercial farms near Beltsville, MD. All squash fruits were of excellent quality and selected for uniformity in size and color. The fruit were then washed with 5% sodium hypochlorite (Chlorox), sliced with a meat slicer (Berkel Inc., Laporte, IN) to a thickness of 5 mm and placed in polystyrene plastic trays (PCA Co, Wheeling, IL) and divided into four groups for germicidal irradiation, hereafter referred to as UV-C irradiation. The first group was irradiated with UV-C for 1 min (total dose 4.93×10^2 Jm^{-2}), the second group for 10 min (total dose 4.93×10^3 Jm^{-2}); and the third group for 20 min (total dose 9.86×10^3 Jm^{-2}). A fourth group was considered as the control group and received no UV-C irradiation. Each tray consisted of eight slices. All squash samples were stored at 5 or 10°C. During the storage period, various chemical and physical analyses were performed at certain intervals to determine fungal, yeast and bacterial populations, sugar and organic acid contents, ethylene production, respiration rate, chilling injury, and UV-C damage.

2.2. UV-C irradiation set-up

UV-C irradiation was provided by unfiltered General Electric 15 watt G15 T8 germicidal lamps. Although small amounts of UV-A (320–400 nm) and UV-B (280–320 nm) radiation were also emitted as well as some visible radiation (400–792 nm), 82% of the total irradiance emitted was in the UV-C (250–280 nm) region (Table 1). Tissue slices of squash fruit were placed in polystyrene trays that were supported on a wide mesh, screened frame and irradiated from the lower and upper surfaces by separate germicidal lamps mounted at a distance of 15 cm. The UV set-up was placed in a fume hood to remove any ozone generated and thereby to eliminate possible confounding factors. As a safety precaution, a timer was used to regulate the duration of UV exposure. Safety goggles, polycarbonate face masks, and protective gloves were also used. UV

Table 1
Spectral irradiance of GE 15 watt germicidal lamp (G15 T8) at a distance of 15 cm from the lamp[a]

Wavelength range (nm)	Integrated irradiance (mW m^{-2})	
	Bare lamp	Polystyrene filtered lamp
250–792	9983	1435
250–400	8597	230
250–320	8414	115
250–280	8220	0.8

[a] Comparative measurements shown for bare lamp and for lamp filtered through a polystyrene tray.

measurements were taken with an Optronic Model 752 UV-VIS spectroradiometer (Optronic Laboratories, Inc., Orlando, FL) to determine the spectral irradiance of the bare lamp and verify the transmission characteristics of the polystyrene trays from 250 to 792 nm. Spectral measurements were taken at the center of the set-up and at 11.5 and 23 cm from each end. Integrated values of spectral irradiance were determined for the wavelength ranges of 250–792, 400–792, 320–400, 280–320, and 250–280 nm. Because of the decline in UV irradiance at the edges of the set-up, samples were only placed on the center portion of the screened frame. Each UV-C treatment was replicated at least three times.

2.3. Respiration measurement

Respiration rates of tissue slices (5 mm thick) were measured as production of CO_2 at 20°C. Carbon dioxide production of squash slices was determined daily until the tissues began to deteriorate. Gas analyses were performed with a Shimadzu gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD).

2.4. Ethylene measurement

Ethylene production of tissue slices was measured at 20°C and expressed as $\mu l\ kg^{-1}\ h^{-1}$. Zucchini squash slices were cut as described above and placed in 1-l jars and incubated for 1 h at 20°C. A total of 5 ml gas was withdrawn at the end of incubation for ethylene determination. Ethylene was analyzed with a Carle (Carle Instruments Inc., Anaheim, CA) hydrogen flame ionization gas chromatograph equipped with an alumina column.

2.5. Analysis of sugars and organic acids

The sugar and organic acid concentrations of the tissue slices were determined in samples stored at 5 and 10°C. A total of 2 g of squash tissue were homogenized with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) in imidazole buffer (20 mM, pH 7.0). The extracts were centrifuged and the supernatants were dried *in vacuo* in derivatizing vials. Procedures described by Li and Schuhmann (1980) were modified for the derivatization of sugars. A known amount of β-phenyl-D-glucopyranoside was included in all samples as an internal standard. A total of 1 ml Trisil reagent (Pierce, Rockford, IL) was mixed vigorously with each sample and then heated at 75°C for 30 min. After silylation, 1 μl (= 1 μg) of each derivatized sample was injected into a Hewlett Packard 5890 gas chromatograph (Hewlett Packard, Palo Alto, CA) equipped with a flame ionization detector and a 25-m crosslinked methyl silicon gum capillary column (0.2-mm ID, 0.33-μm film thickness). Temperatures were as follows: injector 250°C, detector 275°C and column 100–250°C programmed at $10°C\ min^{-1}$ with 0 min initial and 23 min final times. Organic acids were analyzed after extraction with imidazole buffer and purification with a Baker-10 solid phase extraction system. Supernatants from the extract were passed through quaternary amine columns, which were previously conditioned with hexane and methanol. The samples were then eluted from the columns with 0.1 N HCl. The eluates were concentrated to dryness *in vacuo* in derivatized vials. Procedures of derivatization and chromatography for organic acids were the same as those for sugars except that column temperature was programmed from 180 to 250°C at $10°C\ min^{-1}$ with 3 min initial and 12 min final times. The sugars and organic acids were quantified by comparison with derivatized standards.

2.6. Enumeration and differentiation of microorganisms

Squash tissues (20 g) were macerated in 40 ml of sterile peptone water, pH 7.4, with a 400 Lab Stomacher (Seward Medical, London, UK). A sample of each homogenate or appropriate dilution was spread on agar plates using a Spiral Plate System (Autoplate Model 3000, Spiral Biotech, Bethesda, MD). The enumeration of microorganisms was performed by using the following culture media: tryptic soy agar (TSA, Difco Laboratories, Detroit, MI) incubated at 30°C for 24 h for total mesophilic aerobic microorganisms or potato dextrose agar (PDA, Difco) with addition of

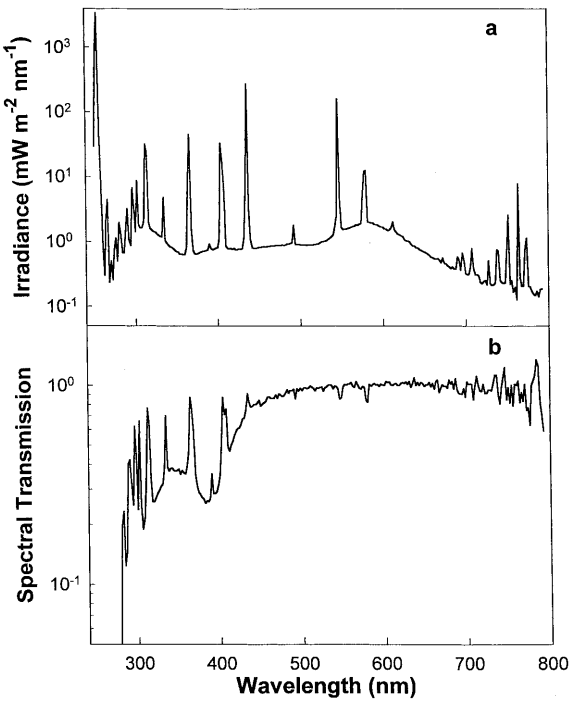

Fig. 1. (a) Spectral energy distribution of bare GE 15-watt germicidal lamp (GE G15 T8). Measurements taken at 15 cm from the lamp, (b) Spectral transmission of a polystyrene tray.

chloramphenicol (500 mmol mol^{-1}) incubated at 30°C for 36 h (Babic et al., 1996) for yeast and molds.

2.7. Statistical analysis

The data were analyzed employing a completely randomized design and PROC-GLM procedure (SAS Institute, 1987).

3. Results

3.1. Spectral irradiance

The comparative spectral irradiance in the 250–792, 250–400, 250–320, and 250–280 nm bandwidths is shown in Table 1. The spectral irradiance of the bare germicidal lamp from 250 to 792 nm is shown in Fig. 1a. The spectral transmission of the polystyrene trays from 250 to 792 nm is shown in Fig. 1b.

3.2. Visual changes in appearance and decay

After 12 days of storage at 10°C, slight UV-C damage was observed as a reddish brown discoloration on the surface of tissues treated for 10 and 20 min. Squash samples stored at 5°C did not show any UV-C-induced damage. Chilling injury was not observed until after 20 days of storage at 5°C. After 20 days, chilling injury was detected on the surface of squash tissues as dried sunken brown spots on the cortex tissue. UV-C irradiation did not affect the degree of chilling injury during storage at 5°C. Samples irradiated for 10 and 20 min had significantly less decay than controls (data not shown). This was true at both 5 and 10°C.

3.3. Effect of UV-C irradiation on microbial population of squash slices

The microbial populations on the surface of *Cucurbita pepo* slices increased during storage. These increases were higher at 10°C than at 5°C. After 10 d, the populations of yeast and fungi were higher in slices of controls and in those given 1 min UV-C than in those exposed to UV-C for 10 or 20 min (Fig. 2). The initial number of microorganisms was 10^2 colony forming units (CFU) g^{-1} for yeast and fungi. At 10°C, the yeast and fungal populations increased more rapidly than at 5°C. After 14 days storage at 10°C, yeast and fungal populations of squash reached 10^7–10^8 CFU g^{-1} in controls and 1 min UV-C irradiated squash, and 10^5–10^4 CFU g^{-1} in 10 and 20 min UV-C irradiated squash. The yeast and fungal populations were found to be lower in 10 and 20 min UV-C irradiated squash than in those of controls and 1 min UV-C irradiated samples at both 5 and 10°C. Similarly, after 5 days of storage, the mesophilic bacterial populations of squash slices were higher in unirradiated controls and in those given 1 min UV-C treatment than in those irradiated for 10 or 20 min at 10°C. These bacterial populations remained relatively constant in all treatments until the 14th day of storage at both 5 and 10°C, but increased sharply thereafter at 5°C in the controls and in those given only 1 min UV-C exposure (Fig. 3).

3.4. Respiration rates

The respiration rates of UV-C irradiated squash slices were higher than those of unirradiated controls (Fig. 4). The increase in the respiration rate of tissue slices was high in samples exposed to UV-C for 20 min, but remained relatively low in control slices.

3.5. Ethylene measurement

Ethylene production of tissue slices showed little or no difference with UV-C exposure (Fig. 5). From day 1 to day 3, all treatments showed a decline in ethylene concentration; but thereafter, the concentration remained constant until day 9.

3.6. Sugar and organic acid concentrations

Fructose, glucose and sucrose were the major soluble sugars and malic acid was the major organic acid in squash. Overall, fructose, glucose and sucrose concentrations of the squash increased at first and then decreased during storage.

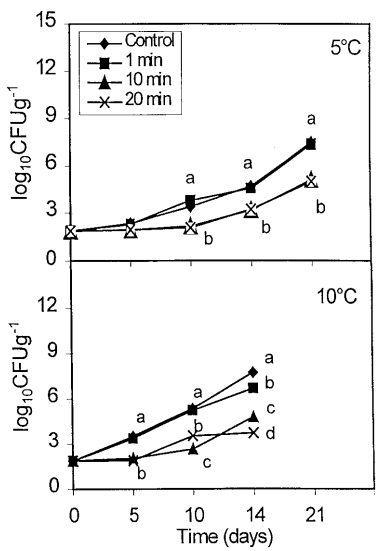

Fig. 2. Influence of UV-C exposure on yeast and fungal populations of *Cucurbita pepo* tissues at 5 and 10°C. Comparisons of means were made within a day for each temperature. Each point is the mean of three samples. Means with different letters are significantly different at the 0.05 level.

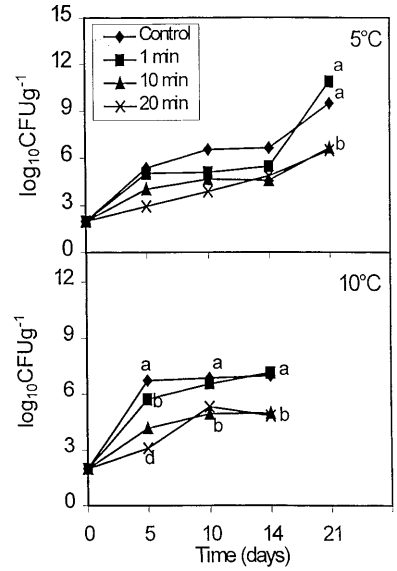

Fig. 3. Influence of UV-C exposure on bacterial populations of *Cucurbita pepo* tissues at 5 and 10°C. Comparisons of means were made within a day for each temperature. Each point is the mean of three samples. Means with different letters are significantly different at the 0.05 level.

There was no appreciable difference in sugar concentration between UV-C irradiated and unirradiated squash slices (Table 2). During storage, the malic acid concentration of the tissue slices showed little change; there was also little difference among UV-C treatments (Table 3).

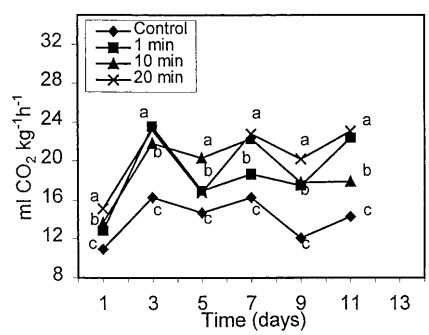

Fig. 4. Influence of different UV-C exposure times on respiration rate of *Cucurbita pepo* tissues at 20°C. Each point is the mean of three samples. Means with different letters are significantly different at the 0.05 level.

Table 2
Effects of storage temperature and UV-C exposure time on carbohydrate concentration of *Cucurbita pepo* tissues after 0, 7, 12 and 18 days[a]

Storage time (days)	Storage temp (°C)	UV-C duration (min)	*Carbohydrate concentration* (mg g^{-1} fresh weight)			
			Fructose	Glucose	Sucrose	Total
0		0	14.6 f	2.7 j	0.4 kl	17.6 j
7	5	0	2.7 m	1.4 lm	2.5 a	6.6 s
		1	4.6 kl	1.6 lm	1.7 efg	7.9 pr
		10	10.6 h	3.0 j	1.4 h	15.0 l
		20	21.2 b	6.2 b	1.4 h	28.7 b
7	10	0	12.6 g	4.2 fg	0.0 m	16.7 k
		1	7.7 i	1.6 l	1.4 h	10.7 mn
		10	23.1 a	7.9 a	0.7 i	31.6 a
		20	4.0 l	2.0 k	1.3 h	7.3 r
12	5	0	18.5 c	4.8 cd	1.9 cde	25.2 cd
		1	18.3 c	4.3 ef	1.7 fg	24.3 d
		10	6.2 j	1.7 kl	1.6 g	9.5 n
		20	4.9 k	1.2 m	1.9 cd	8.0 p
12	10	0	14.0 f	4.9 c	0.5 j	19.4 i
		1	17.4 d	5.0 c	0.5 j	22.9 ef
		10	14.7 f	4.2 f	0.5 jk	19.4 i
		20	16.9 e	3.6 hi	1.9 cde	21.6 g
18	5	0	17.4 d	4.6 de	1.8 def	23.8 de
		1	16.6 e	3.5 I	2.0 c	22.1 f
		10	14.7 f	2.7 j	2.2 b	19.6 i
		20	18.0 cd	3.9 gh	1.6 g	23.5 e
18	10	0	16.5 e	3.7 hi	0.5 jk	20.6 h
		1	7.3 i	2.0 k	0.3 l	9.6 n
		10	7.3 i	1.6 kl	0.0 m	8.9 o
		20	5.3 k	1.7 kl	0.0 m	7.0 r

[a] Mean separation by Duncan's multiple range test. Values followed by different letters within a column are significantly different at the 0.05 level.

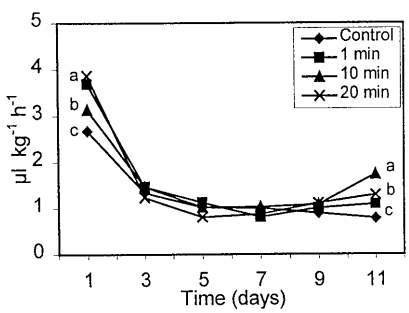

Fig. 5. Influence of different UV-C exposure times on ethylene production of *Cucurbita pepo* tissues at 20°C. Each point is the mean of three samples. Means with different letters are significantly different at the 0.05 level.

4. Discussion

Although many studies have demonstrated the efficacy of using germicidal UV lamps for extending shelf life of fruits and vegetables, most have utilized intact produce (Wilson et al., 1997). Our results in using UV-C irradiation to reduce microbial populations on the surface of *Cucurbita pepo* slices are consistent with those reported for low UV-C doses in reducing postharvest diseases of intact fruits and vegetables such as sweetpotato (*Ipomea batatas* L.) (Stevens et al., 1990), peach (*Prunus persica* (L.) Batsch.) and apple (*Malus* × *domestica* Borkh.) (Stevens et al., 1996), table grapes (*Vitis vinifera* L.) (Nigro et al., 1998), carrot (*Daucus carota* L.) (Mercier and Arul, 1993), tomato (*Lycopersicon esculentum* Mill.)

Table 3
Effects of storage temperature and UV-C exposure time on organic acid concentration of *Cucurbita pepo* tissues after 0, 7, 12 and 18 days[a]

Storage time (days)	Storage temp (°C)	UV-C duration (min)	*Organic acid concentration* (mg g^{-1} fresh mass)
			Malic acid
0		0	0.84 de
7	5	0	0.52 ij
		1	0.76 ef
		10	0.26 m
		20	0.88 d
7	10	0	1.12 c
		1	0.37 kl
		10	0.54 hij
		20	0.38 kl
12	5	0	0.58 hi
		1	1.32 a
		10	0.89 d
		20	0.45 jk
12	10	0	0.56 hij
		1	0.05 n
		10	0.00 n
		20	1.23 ab
18	5	0	0.55 hij
		1	0.77 ef
		10	0.84 de
		20	0.53 hij
18	10	0	0.32 lm
		1	0.69 fg
		10	1.21 bc
		20	0.64 gh

[a] Mean separation by Duncan's multiple range test. Values followed by different letters within a column are significantly different at the 0.05 level.

(Liu et al., 1993) and grapefruit (*Citrus paradisi* Macfad.) (Droby et al., 1993).

The application of UV-C has been reported as being effective in reducing naturally infected green mold rot (*P. digitatum* Sacc.), alternaria rot (*Alternaria citri* Ell. & Pierce), as well as sour rot (*Geotrichum candidum* Lk. ex Pers.) of Dancy tangerines (*Citrus reticulata* Blanco.) 17 days after irradiation (Leach, 1971; Stevens et al., 1996). The decrease in microbial population in *Cucurbita pepo* samples irradiated for 10 or 20 min could certainly explain the concomitant reduction in decay observed. Since UV-C radiation is known to cause DNA damage including the formation of pyrimidine dimers (Mitchell et al., 1992; Slieman and Nicholson, 2000) and single-strand and double-strand breaks (Slieman and Nicholson, 2000), and induction of chromosomal aberrations (Cieminis et al., 1987), the increased mortality of microorganisms with increased UV-C is not unexpected. Although it would be useful to know what the acceptable levels of surface populations of microorganisms are for this species, such guidelines have not been established. Nevertheless, the reduction in microorganisms by UV-C irradiation could have ancillary health benefits.

The reduction in decay in the irradiated commodities might also be related to the increase in decay-resistance of tissues due to the accumulation of antifungal compounds. Increased formation of phytoalexin (Mercier et al., 1993) and pisatin and phenylalanine ammonia-lyase (Hadwiger and Shwochau, 1971) by UV irradiation has been reported. Increased accumulation of sco-

parone and scopoletin in oranges (*Citrus sinensis* (L.) Obsek) has also been implicated in the UV-C induced resistance to postharvest decay (D'hallewin et al., 1999). UV-C irradiation might also stimulate the activity of lignifying enzymes and enhance the protection against pathogenic invasion (Liu et al., 1991, 1993).

Examination of Fig. 1a, b, and Table 1 shows that the UV cutoff of the polystyrene trays is approximately 280 nm, indicating that UV-C exposure to the squash slices was only provided by the upper lamp fixture. The appearance of reddish brown lesions on the surface of UV-C irradiated tissues suggests the accumulation of phenolic compounds, which are known to be induced by UV radiation (Habeck and Curtis, 1974; Glaßgen et al., 1998). The fact that no differences were found in the concentrations of soluble sugars among different UV-C treatments was probably due to the large amount of variability. Similarly, this could also explain the poor relationship between ethylene production and microbial activity following UV-C irradiation.

Our findings confirm the hypothesis that brief periods of UV-C exposure are effective in delaying senescence and deterioration in *Cucurbita pepo* tissues by reducing the microbial population and thereby improve storage quality. The precise physiological and biochemical processes that are altered by germicidal UV radiation remain to be elucidated.

Acknowledgements

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