1-Methylcyclopropene and storage temperature influence responses of 'Gala' apple fruit to gamma irradiation

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Abstract

'Gala' apple (*Malus x domestica* Borkh.) fruit pre-treated with 0.5 μL L^{-1} 1-methylcyclopropene (MCP) or air (control) for 12 h at 20 °C were exposed to gamma radiation at doses of 0, 0.44, 0.88 or 1.32 kGy at 23 °C. The fruit were then stored at 20 °C for 3 weeks or at 0 °C for 8 weeks plus 7 days at 20 °C. Fruit treated with MCP had higher firmness and titratable acidity (TA) than control fruit after storage at either temperature. During the post-irradiation storage at 20 °C, irradiation promoted respiration of MCP-treated fruit throughout the 3-week period but had no consistent effect on respiration of control fruit. Fruit firmness and TA decreased with increased radiation dose after 3 weeks storage at 20 °C regardless of MCP treatment. Compared to non-irradiated fruit, irradiated fruit had lower TA and similar firmness after storage at 0 °C for 8 weeks plus 7 days at 20 °C. Some irradiated fruit stored at 20 °C for 3 weeks developed internal browning, and MCP-treated fruit had more injury than control fruit. Storage at 0 °C after irradiation greatly reduced development of internal browning. Production of volatile esters, alcohols and 6-methyl-5-hepten-2-one by fruit stored at 0 °C was reduced. The magnitude of reduction directly increased with radiation dose. It appears that some responses of apple fruit to gamma radiation are influenced by ethylene action and post-irradiation storage temperature. Published by Elsevier Science B.V.

Keywords: Apple; Firmness; Titratable acidity; Irradiation; 1-methylcyclopropene; Volatile compounds

1. Introduction

Ionizing radiation inhibits ripening and senescence, prolongs shelf life, and reduces spoilage of many fruits (Thomas, 1986). Interest in the use of irradiation has increased in recent years due to its effectiveness for insect disinfestation (Hallman, 1999) and enhanced food safety (Thayer and Rajkowski, 1999). Successful control with less than 1

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kGy irradiation has been demonstrated on a number of insects important in postharvest handling and marketing of fresh horticultural crops. Although irradiation at 0.2 kGy was effective in preventing codling moth adult emergence from larvae in apple fruit (Burditt, 1982), the dose that some fruit received could be tripled in some commercial situations due to dose variation among the fruit (Hallman, 1999). Irradiation has been approved for many years by the USDA and FDA for use on fruits and vegetables at doses up to 1.0 kGy for insect disinfestation and shelf-life extension, however, commercial use of irradiation technology to extend postharvest life of fruits has been limited, in part, due to its adverse effects on fruit quality (Maxie and Abdel-Kader, 1966; Maxie et al., 1971).

The principal adverse effects of irradiation are undesirable changes in texture, loss of acidity and development of off-flavor and injury (Maxie and Abdel-Kader, 1966). Irradiation at doses above 0.1 kGy enhances apple fruit softening although irradiated fruit soften at a slower rate during storage at 0 °C (Massey et al., 1964). Apples exposed to 0.4 kGy gamma radiation were softer than controls after storage at 23.3 °C (Smock and Sparrow, 1957). Many factors influence fruit responses to irradiation, including fruit maturity, cultivar, storage temperature, and the use of controlled atmosphere storage (Maxie and Abdel-Kader, 1966; Miller and McDonald, 1999). The ethylene action inhibitor, 1-methylcyclopropene (MCP) (Sisler and Blankenship, 1996; Sisler and Serek, 1997), slows apple fruit ripening including the loss of firmness and titratable acidity (TA) (Fan et al., 1999; Watkins et al., 2000). It is unclear whether loss of firmness and TA, and development of injury caused by irradiation can be reduced or eliminated by MCP.

Information concerning impact of irradiation on the production of volatile compounds that contribute to aroma of whole fruit is limited. Volatile compounds are an important quality attribute of apple fruit. The largest proportion of volatiles produced by apples are esters, alcohols, and aldehydes. Production of esters and alcohols increases during apple fruit ripening, contributing, in part, to the development of characteristic aroma (Brown et al., 1966). Tobback et al. (1973) have measured production of 13 volatile compounds from 'Boskoop' apple fruit following gamma radiation at 0.5, 2 or 5 kGy. Alcohol production was either reduced or stimulated depending on the alcohols involved although only semi-quantitative data were presented. Production of most volatile compounds including ethylene was inhibited by irradiation during storage at 20 °C (Fan et al., 2001).

The objective of this study is to examine the effect of MCP and storage temperature on quality of irradiated apple fruit.

2. Materials and methods

2.1. Fruit source

'Gala' apple fruit (*Malus x domestica* Borkh.) were harvested at commercial maturity from a research orchard near Wenatchee, WA.

2.2. MCP treatment, irradiation

Fruit were treated on the day of harvest with MCP generated from Ethylbloc (Floralife Inc, Walterboro, SC). The treatment was applied at 0.5 μL L^{-1} for 12 h at 20 °C in a sealed 230 L steel chamber. Control fruit were treated similarly but without MCP. Following MCP treatment, fruit were held in air at 20 °C overnight, then transported to an irradiation facility and exposed to gamma radiation doses of 0, 0.44, 0.88 or 1.32 kGy at 23 °C. Irradiation was carried out in a GammaBeam 650 (Nordion International Inc, Kanata, Ont., Canada) facility located at the Battelle Pacific Northwest National Laboratory in Richland, WA. The GammaBeam facility contains approximately 14 000 curies of cobalt-60. All samples were irradiated in a single geometric configuration at a dose rate of approximately 240 Gy h^{-1} and calibrated at the preferred geometry. The actual dose distribution was determined using alanine dosimeters (Bruker Instruments Inc., Billerica, MA). The ratio of maximum to minimum dose received was 1.4. Details of irradiation and MCP treatment were described earlier (Fan et al., 2001).

2.3. Storage, measurement of respiration and quality

After treatments, fruit were stored for 3 weeks at 20 °C, or 8 weeks at 0 °C plus 7 days at 20 °C. Respiration was measured on the day of irradiation and 1, 3, 7, 14 and 21 days after irradiation during storage at 20 °C. Fruit were placed into 4-L glass jars sealed with Teflon lids with two gas ports. There were five fruit in each jar and four jars (replicates) in each treatment. Purified compressed air flowed at 6 L h^{-1} through the jars and gas samples were collected from each jar outlet. CO_2 in the gas sample was analyzed using gas chromatography as described previously (Fan and Mattheis, 1999). Fruit firmness, TA, soluble solids content (SSC), internal radiation damage, and surface color were measured after 21 days storage at 20 °C and after 8 weeks storage at 0 °C plus 7 days at 20 °C. Measurements of firmness, TA, SSC, surface color and volatile composition were as described previously (Fan et al., 1998). Radiation damage was subjectively assessed using a scale of 1–4 after fruit were halved transequatorially: (1) free of radiation damage; (2) slight, light diffused brown areas underneath the peel and/or adjacent to the vascular bundles; (3) medium: 1–4 dark brown spots underneath the peel and/or adjacent to the vascular bundles; and (4) severe, more than five dark brown spots underneath the peel and adjacent to the vascular bundles, spots tended to be connected.

2.4. Measurement of volatile compounds

Volatile compounds were measured (Mattheis et al., 1991) after 8 weeks storage at 0°C plus 1 or 7 days at 20 °C. Volatile compounds in the outlet gas of jars described above were adsorbed onto 50 mg of 30–50 mesh Tenax TA (Alltech Associates, Deerfield, IL) packed in glass tubing (17.5 cm H 0.4 cm i.d.). The Tenax traps were desorbed at 250 °C for 3 min using a Tekmar 6000 AeroTrap Autosampler (Tekmar Co., Cincinnati, OH). The desorbed sample compounds were condensed at − 120 °C, then the cryofocusing module was flash heated to 250 °C and He carrier gas carried the analytes into a Hewlett-Packard 5890A/5971A GC-MSD (Agilent Technologies, Palo Alto, CA) equipped with a DB-Wax column (J&W Scientific, Folsom, CA) (60 m H 0.25 mm i.d., 0.25 μm film thickness). Compounds were identified by comparison of spectra of sample compounds with those contained in the Wiley-NBS library and by comparing retention indices of sample compounds and standards. Compounds were quantified using selected ion monitoring for base peaks, and quantitative values were calculated using response factors generated with standards. A total of 48 volatile compounds were monitored, including esters, alcohols, aldehydes, acids, and ketones.

2.5. Statistical analysis

The experimental design was factorial with two factors: MCP and radiation dose. Data were subjected to statistical analysis using SAS ver. 6.12 (SAS Institute, Raleigh, NC). The effect of radiation dose was analyzed using orthogonal comparisons. Significance of polynomials was calculated using the Contrast statement of the GLM procedure of the SAS System. The effect of MCP and the interaction of MCP with radiation dose were analyzed using the GLM procedure.

3. Results

3.1. Fruit quality at harvest

The mean internal ethylene concentration of fruit at harvest was 5.28 μL L^{-1} (ranging from 0.04 to 43.4 μL L^{-1}), indicating the onset of the climacteric had occurred in some fruit. At the time of irradiation, fruit had a firmness of 82.6 ± 8.7 N (average ± standard deviation); starch index of 3.2 ± 1.3 on a scale of 1–6; SSC of 13.5 ± 0.4%, TA of 48.2 ± 4.3 mmol H^{+} L^{-1} juice, and chroma of 37.5 ± 4.2.

3.2. Storage at 20°C

Respiration rate of control fruit was not consistently affected by irradiation (Fig. 1). Respiration rate of MCP-treated fruit decreased after MCP treatment and remained at 0.2–0.4 mmol kg^{-1}

h^{-1} for 7 days before rising slowly to levels that were still lower than that in controls. Fruit treated with 1.32 kGy were an exception to this pattern. Irradiation increased respiration rate of MCP-treated fruit throughout the 21 days at 20 °C, and fruit irradiated with 1.32 kGy had the highest respiration rate.

Fruit treated with MCP had higher firmness, SSC and TA and lower chroma than the controls after 21 days storage at 20 °C (Table 1). Firmness decreased linearly with increased radiation dose in both MCP-treated and control fruit. MCP treatment did not eliminate the loss in firmness caused by irradiation, but fruit treated with MCP followed by irradiation had significantly higher firmness and TA than irradiated fruit without previous MCP exposure. SSC was not affected by irradiation except that control fruit irradiated with 0.88 kGy had higher SSC than those treated with 0.44 kGy (data not shown). TA and chroma decreased linearly with increased radiation dose. There was no difference in hue value (data not shown). Irradiation lowered the chroma values, suggesting that irradiated fruit were darker in surface color.

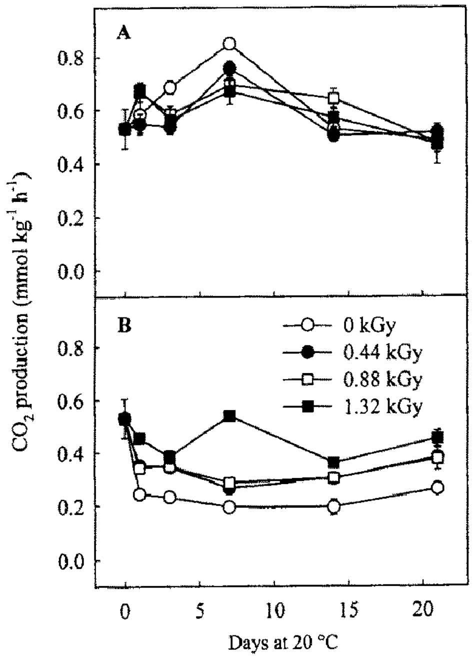

Fig. 1. Irradiation effect on respiration rate of control (A); and MCP-treated (B) apple fruit. Mean standard deviations are indicated, and differences between means that exceed the mean standard deviations were always significant when analyzed using the SAS GLM procedure.

Irradiated fruit developed internal injury (Table 1) with symptoms expressed as diffuse browning of the outer mesocarp and/or tissue adjacent to the vascular bundles. When injury was severe, brown spots appeared in both the outer mesocarp and tissues adjacent to the vascular bundles and browning could be observed externally. The severity of radiation injury increased linearly with increased radiation dose. MCP treatment resulted in development of more severe radiation injury.

3.3. Storage at 0 °C

MCP-treated fruit had higher firmness and TA and similar SSC or chroma compared to control fruit after 8 weeks storage at 0 °C plus 7 days at 20 °C (Table 2). Irradiation had no effect on firmness, SSC or chroma. Loss of TA increased linearly with radiation dose in both MCP-treated and control fruit. Irradiation at 1.32 kGy induced some injury in control fruit but much less injury was observed compared to fruit stored at 20 °C following irradiation.

MCP treatment resulted in decreased production of volatile compounds including esters and alcohols (Table 3). One day at 20 °C after 8 weeks storage, production of esters, alcohols, and 6-methyl-5-hepten-2-one (MHO) by both MCP-treated and control fruit was reduced by irradiation; however, aldehyde production was not significantly altered. The reduction in volatile production increased with radiation dose. After an additional 7 days at 20 °C, production of esters, alcohols, and MHO by control fruit decreased with increased radiation dose. Production of aldehydes was stimulated several times by the 1.32 kGy radiation. Irradiation-promoted aldehydes included pentanal, heptanal, hexanal, octanal, nonanal, and decanal. Butanal and benzaldehyde were not affected (data not shown). Irradiation only reduced production of esters by MCP-treated fruit. Fruit treated with 0.44 kGy had a similar production rate of total volatile compounds compared to non-irradiated controls after 8 weeks at 0 °C plus 7 days at 20 °C.

Table 1
Irradiation and MCP effect on firmness, titratable acidity (TA), surface color (chroma), and internal radiation damage of 'Gala' apples

Dose (kGy)	Firmness (N)	TA (mmol H^+ L^{-1})	Chroma	Internal damage (1–4)
Control fruit				
0	62.7	42.2	48.7	1.0
0.44	59.3	37.5	47.3	1.2
0.88	59.5	40.0	45.8	1.4
1.32	51.8	38.9	40.9	2.4
Radiation				
Linear	**	*	***	***
Quadratic	NS	NS	NS	*
Cubic	NS	NS	NS	**
MCP treated fruit				
0	83.3	47.9	42.7	1.0
0.44	75.3	44.3	42.8	1.6
0.88	72.3	42.4	33.6	2.0
1.32	61.7	41.9	32.6	3.1
Radiation				
Linear	***	**	*	***
Quadratic	NS	NS	NS	NS
Cubic	***	*	***	***
MCP	***	***	***	*
MCP × radiation	**	NS	NS	NS

Fruit were irradiated with 0, 0.44, 0.88 and 1.32 kGy gamma radiation after treatment with air (control) or 0.5 μL L^{-1} MCP, then stored in air for 3 weeks at 20 °C. NS, *, **, ***, nonsignificant or significant at $P<0.05$, 0.01, or 0.001, respectively.

4. Discussion

Fruit maturity and radiation dose have an influence on fruit respiratory response to irradiation. Irradiation effect on respiration of 'Bartlett' pears is reduced if the fruit are treated near the climacteric peak rather than preclimacteric (Romani et al., 1961; Maxie et al., 1966). Respiration of 'Rome Beauty' apples is reduced by irradiation treatment at 0.05 kGy but stimulated at higher doses (Massey et al., 1964). Fruit used in the present study had entered the climacteric stage of development, but a climacteric pattern of respiration was still observed in control fruit. Irradiation at 0.88 and 1.32 kGy slightly stimulated respiration of control fruit 1 day after irradiation but reduced the respiration rate from 3 to 7 days (Fig. 1A). These results were in agreement with those from tomato fruit in which irradiation stimulated respiration of tomatoes stored at 20 °C between 1 and 3 days after irradiation but inhibited it from day 4 to 12 (Abdel-Kader et al., 1968). Respiration rate of MCP-treated fruit was stimulated by irradiation throughout the investigation period (Fig. 1). The promotion of respiration by irradiation has been observed mainly in non-climacteric fruit (Maxie and Abdel-Kader, 1966). It appears that MCP-treated-fruit respond similarly to irradiation as non-climacteric fruit.

Fernandes and Clarke (1962) reported that irradiated apples seemed to taste sweeter than non-irradiated fruit and the amount of malic acid was much less in irradiated apples. Maxie et al. (1964) reported a marked decrease in total acidity in irradiated lemon fruit. In the present study, we observed that irradiation reduced TA but had little effect on SSC, therefore, the sweet taste of irradiated fruit may result from an increase in the SSC/TA ratio.

Irradiated fruit had lower firmness after storage at 20 °C and lower TA after storage at both 20 and 0 °C compared to controls. However, firmness was similar after 8 weeks storage at 0 °C plus 7 days at 20 °C. It appears that storage durations and storage conditions have an impact on the irradiation effect. Many earlier reports showed irradiation promotes firmness loss of apple fruit measured immediately after irradiation, but irradiated fruit softens at a slower rate during cold storage (Massey et al., 1964; Al-Bachir, 1999). 'Delicious' apples irradiated at 1 kGy were softer and had lower TA immediately after irradiation, however, after 11 months of controlled atmosphere storage, all the apples had similar TA and firmness (Olsen et al., 1989).

Storage temperature and MCP treatment have a profound influence on apple fruit responses to irradiation. Respiration of control fruit was not consistently influenced by irradiation; but respiration of MCP-treated fruit was enhanced throughout the 21 days at 20°C (Fig. 1). Irradiation inhibited ethylene production in control fruit, but had little effect in MCP-treated fruit except that a stimulation effect occurred 1 day following irradiation (Fan et al., 2001). Irradiation-induced injury was observed primarily in fruit stored at 20 °C. Treatment with MCP prior to irradiation increased sensitivity to radiation injury when fruit were stored at 20 °C. Fruit maturity and storage temperature have been found to influence irradiation-induced injury of papaya fruit (Miller and McDonald, 1999). Peel scald developed on irradiated fruit stored at 10 °C, but delaying cold storage for 12 h after irradiation treatment inhibited the development of scald (Paull, 1996). Our results showed that storage at 0 °C after irradiation almost eliminated the development of radiation injury of apple fruit.

The loss of firmness and TA was observed in both control and MCP-treated fruit stored at 20°C, indicating that the texture change and acidity loss caused by radiation is unlikely to be associated with ethylene action. The texture change induced by irradiation may result from pectin degradation caused by direct action of radiation (Zhao et al., 1996). The loss of firmness

Table 2
Irradiation and MCP effect on firmness, titratable acidity (TA), surface color (chroma), and internal radiation damage of 'Gala' apple

Dose (kGy)	Firmness (N)	TA (mmol H^+ L^{-1})	Chroma	Internal damage (1–4)
Control fruit				
0	64.2	39.6	44.8	1.0
0.44	66.1	35.2	44.3	1.0
0.88	62.3	35.5	45.6	1.0
1.32	63.2	36.3	42.2	1.1
Radiation				
Linear	NS	*	NS	NS
Quadratic	NS	*	NS	NS
Cubic	NS	NS	NS	NS
MCP treated fruit				
0	76.0	49.3	44.6	1.0
0.44	75.8	45.2	44.0	1.0
0.88	76.3	42.7	42.8	1.0
1.32	73.5	40.9	42.1	1.0
Radiation				
Linear	NS	***	NS	NS
Quadratic	NS	NS	NS	NS
Cubic	NS	**	NS	NS
MCP	***	***	NS	NS
MCP × radiation	NS	*	NS	NS

Fruit were irradiated with 0, 0.44, 0.88 and 1.32 kGy gamma radiation after treated with air (control) or 0.5 μL L^{-1} MCP, then stored in air for 8 weeks at 0 °C plus 1 week at 20°C. NS, *, **, ***, nonsignificant or significant at $P<0.05$, 0.01, or 0.001, respectively.

Table 3
Irradiation and MCP effect on production (μmol kg^{-1} h^{-1}) of volatile esters, aldehydes, alcohols and 6-methyl-5-hepten-2-one (MHO) of 'Gala' apple

Dose (kGy)	Esters		Alcohols		Aldehydes		MHO	
	Control	MCP	Control	MCP	Control	MCP	Control	MCP
1 day at 20°C after storage								
0	3094.7	1334.4	252.2	126.7	140.4	90.9	3.8	3.7
0.44	1487.8	294.3	167.1	61.3	117.3	99.9	2.1	2.8
0.88	1020.5	162.6	166.6	45.5	93.8	64.3	2.6	0.9
1.32	339.8	64.8	69.8	32.1	90.2	67.9	2.4	0.9
Radiation								
Linear	***	***	***	***	NS	NS	***	**
Quadratic	***	NS	*	NS	NS	*	NS	
Cubic	*	*	NS	NS	NS	NS	**	NS
MCP	***	***	NS	NS				
MCP × radiation	***	NS	NS	NS				
7 days at 20°C after storage								
0	2623.7	587.3	208.0	50.4	40.7	67.6	3.5	2.3
0.44	2158.2	269.3	178.8	34.7	69.1	122.2	3.3	3.7
0.88	1189.4	79.4	108.5	44.7	61.4	91.4	2.4	1.6
1.32	389.9	70.8	59.4	40.7	145.2	76.1	1.6	1.9
Radiation								
Linear	***	***	**	NS	***	NS	*	NS
Quadratic	NS	*	NS	NS	NS	NS	NS	NS
Cubic	NS	NS	NS	NS	NS	NS	NS	NS
MCP	***	***	NS	NS				
MCP × radiation	***	***	NS	NS				

Fruit were irradiated with 0, 0.44, 0.88 and 1.32 kGy gamma radiation after treatment with air (control) or 0.5 μL L^{-1} MCP, then stored in air for 8 weeks at 0 °C. Volatile production of fruit was measured after 1 and 7 days at 20 °C following removal from storage at 0 °C. NS, *, **, ***, nonsignificant or significant at $P<0.05$, 0.01, or 0.001, respectively.

was not observed when apple fruit was stored at 0°C but the loss of acidity was still evident. Fruit treated with MCP followed by irradiation had higher firmness and TA compared to irradiated fruit without MCP treatment, indicating MCP treatment can be combined with irradiation and proper storage temperature management to minimize loss of firmness and TA as well as damage caused by irradiation.

The low TA in irradiated fruit may result from accelerated rates of respiration and metabolism. This may also be the case for MCP-treated fruit in which irradiation promoted respiration throughout the 21 days at 20°C. Respiration of control fruit was, however, not consistently altered by irradiation. The low TA may be related to the development of irradiation injury (Fernandes and Clarke, 1962).

Apple sensitivity to irradiation and symptoms of injury vary with cultivars. In 'Rhode Island Greening' apples, the internal symptoms are similar to mealy breakdown (Smock and Sparrow, 1957). In 'Rome Beauty' apples, core browning can develop after irradiation (Massey et al., 1964). 'Cortland' and 'McIntosh' apples appear to be less sensitive to irradiation (Massey et al., 1964). Injury symptoms in 'Gala' apples were primarily internal browning. Irradiation injury may also contribute to low firmness and TA of fruit stored at 20 °C.

Both MCP treatment and irradiation inhibited production of similar volatile compounds. MCP has been known to inhibit production of many volatile compounds in climacteric fruits (Abdi et al., 1998; Golding et al., 1998; Fan et al., 1999).

An alcoholic flavor developed in 'Cortland' apple fruit irradiated at 1 kGy (Massey et al., 1964), and production of some alcohols by 'Boskoop' apple fruit was stimulated by irradiation (Toback et al. (1973). We did not observe any irradiation-induced alcohol production in control or MCP-treated fruit. In fact, irradiation decreased production of volatile alcohols in all apples in our study. Irradiation did, however, stimulate aldehyde production by control fruit irradiated at 1.32 kGy after 8 weeks storage at 0 °C plus 7 days at 20°C. It is unclear whether this increase in aldehyde production has any effect on apple flavor.

Production of MHO decreased following irradiation. Production of MHO has been associated with the development of superficial scald in apple fruit (Mir et al., 1999). Irradiation reduces development of superficial scald (Massey et al., 1964; Al-Bachir, 1999). Although we did not observe any superficial scald on 'Gala' apples due to the fruit's lack of susceptibility to the disorder, reduced production of MHO following irradiation may contribute to the lack of scald development on irradiated apples.

In summary, respiration rate of MCP-treated fruit increased following irradiation. Irradiation stimulated firmness loss of fruit stored at 20 °C but not when fruit were stored at 0 °C. Irradiation promoted TA loss of fruit stored at either 0 or 20 °C, and the amount of TA loss increased with radiation dose, which is independent of ethylene action. Irradiation inhibited production of many volatile compounds after storage at 0 °C, and inhibition also increased with radiation dose. Production of volatile esters was most inhibited followed by alcohols and MHO. Production rate of volatile compounds by fruit irradiated with 0.44 kGy was comparable to untreated control fruit after 8 weeks at 0 °C plus 7 days at 20 °C, indicating that effects of low dose radiation on volatile production was temporary. Internal injury developed in irradiated fruit stored at 20 °C, and treatment with MCP increased the sensitivity of fruit to radiation injury. Fruit stored at 0 °C developed much less injury, suggesting that irradiation can be combined with MCP and proper storage temperature to maximize firmness and TA retention and minimize development of radiation damage.

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