Impacts of Ionizing Radiation on Volatile Production by Ripening Gala Apple Fruit

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Apple (*Malus x domestica* Borkh., cv. Gala) fruit treated with 0.5 $\mu L \cdot L^{-1}$ 1-methylcyclopropene (MCP) or air (non-MCP) 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 and then stored at 20 °C. Production of volatile compounds was measured on the day of irradiation and 1, 3, 7, 14, and 21 days after irradiation. Both MCP treatment and irradiation inhibited ethylene production. MCP treatment reduced production of all volatile esters and alcohols detected, whereas irradiation inhibited production of most, but not all, esters and some alcohols by non-MCP-treated fruit. The inhibition of volatile production following irradiation increased with dose. Production of methyl and propyl esters was inhibited more than that of other esters following irradiation or MCP treatment. The impact of irradiation on production of esters and alcohols by MCP-treated fruit was minimal. Non-MCP-treated fruit irradiated at 0.44 kGy produced the most esters during the 21-day period at 20 °C following irradiation, and the ester production rate in these fruit was comparable to that of the nonirradiated fruit 21 days after irradiation. Fruit treated with doses higher than 0.44 kGy did not recover their ability to produce volatile compounds. These results indicate both MCP and ionizing radiation inhibit production of many aroma compounds produced by ripening apple fruit.

Keywords: *Apple; ethylene; irradiation; 1-methylcyclopropene; volatile production*

INTRODUCTION

Exposure to ionizing radiation inhibits ripening and senescence, prolongs shelf life, and reduces spoilage of many fruits and vegetables (*1*). Commercial use of irradiation technology to extend postharvest life of fruits and vegetables has been limited because of its adverse effects on some fruit quality attributes and availability of more economical alternatives (*2, 3*). In recent years, interest in the use of irradiation has increased because of its effectiveness for insect disinfestation (*4*) and enhanced food safety (*5*). Irradiation has been approved by the U.S. Department of Agriculture (USDA) and the U.S. Food and Drug Administration (FDA) for use on fruits and vegetables at doses up to 1.0 kGy for insect disinfestation and shelf life extension. A petition filed by a coalition led by the National Food Processors Association proposes that use of ionizing irradiation up to 4.5 kGy be allowed for control of foodborne pathogens and extension of shelf life in fresh and preprocessed fruits and vegetables (*6*).

Many studies have been conducted to identify effects of irradiation on fruit texture, color, and other quality parameters (*2, 7, 8*). However, information concerning impacts of irradiation on the production of volatile compounds that contribute to the aroma of whole fruit is limited. Development of off-odors and reduced aroma following irradiation of fruits has been indicated in several reports (*2, 7, 9*).

Production of some volatile compounds by tomato and mango fruit ceased after irradiation at 1.0 kGy or higher doses, but doses lower than 1.0 kGy delayed the onset of production of ripening-related volatile compounds (*10* and *11*). When Bartlett pear fruit were exposed to gamma radiation at the mature green stage and allowed to ripen, 2.5 kGy reduced production of many volatile compounds, but the fruit developed characteristic flavor when ripe. Pears irradiated at doses 5 kGy or above failed to develop characteristic flavor (*12*). The amount of 8-carbon volatile compounds produced by fresh mushrooms decreased with increased irradiation dose (*13*). Irradiation at 0.05 kGy did not impact volatile production by ginger rhizomes immediately after treatment. After 3 months of storage, the quantities of some major volatile compounds such as α-zingiberence, α-bergamotene, neral, geranial, and α-curcumene were, however, lower in irradiated rhizomes when compared to those in nonirradiated rhizomes (*14*). Irradiation induces only quantitative differences in volatile production by citrus fruit (*15* and *16*).

Volatile compounds are an important quality attribute of apple fruit. The volatiles produced by apples in the largest proportion are esters, alcohols, and aldehydes. Production of volatile compounds by apples is dependent on the stage of fruit development. Production of esters and alcohols increases during apple fruit ripening, contributing in part to development of characteristic aroma (*17*). Tobback et al. (*9*) measured production of 13 volatile compounds from Boskoop apple fruit following gamma irradiation at 0.5, 2, or 5 kGy. Alcohol

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10.1021/jf0008994
Published on Web 12/06/2000

production was either reduced or stimulated depending on the types of alcohols although only semiquantitative data were presented.

Ethylene regulates production of volatile compounds during climacteric fruit ripening (*18*−*20*). The impact of gamma radiation exposure on apple fruit ethylene production is dose dependent. Doses less than 0.5 kGy stimulate ethylene production, but doses of 0.6 kGy or more result in decreased ethylene production (*21*). The ethylene action inhibitor 1-methylcyclopropene (MCP) (*22* and *23*) also inhibits production of ethylene and volatile compounds by apple fruit (*20*). It is unknown whether the irradiation effects on production of ethylene and volatile compounds result from inhibition of ethylene action.

The objectives of this study were to characterize production of ethylene and volatiles by Gala apple fruit following treatment with ionizing radiation and to evaluate whether the effects of irradiation are dependent on ethylene action.

MATERIALS AND METHODS

Apple (*Malus x domestica* Borkh. cv. Gala) fruit at commercial maturity were harvested from a research orchard near Wenatchee, WA. Fruit were treated the day of harvest with MCP generated using Ethylbloc (Floralife Inc, Walterboro, SC). The treatment was applied at 0.5 $\mu L \cdot L^{-1}$ MCP for 12 h at 20 °C in a sealed 230-L steel chamber. Control fruit were treated similarly but without MCP. Following MCP treatment, the fruit were held in air at 20 °C overnight, then transported to the irradiation facility and exposed to gamma radiation at doses of 0, 0.44 (0.35 to 0.49), 0.88 (0.70−0.98), or 1.32 kGy (1.05 to 1.47) at 23 °C.

Gamma Irradiation. Irradiation was carried out in a GammaBeam 650 (Nordion International Inc, Kanata, ON) 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 $Gy \cdot h^{-1}$ and calibrated at the preferred geometry. Following the calibration, boxes (30 × 31 × 50 cm), each containing 4 trays of apples, were placed upright on the irradiation table 50 cm from the centerline of the source cluster. Apples on the top two trays were non-MCP-treated fruit and those on the bottom two trays had previously been treated with MCP. There were 40 apples for each treatment (20 fruit per tray). Irradiation doses were obtained by varying exposure duration to the cobalt-60 source. Halfway through each treatment period, the boxes were rotated 180° to ensure a uniform radiation dose. Actual dose distribution was determined using alanine dosimeters (Bruker Instruments Inc., Billerica, MA) in plastic vials inserted into fruit after removal of core tissue. These fruit were used only to establish the radiation doses. The free radical signal induced in response to irradiation was measured by inserting the alanine dosimeters into a 104 electron paramagnetic resonance instrument (Bruker Instruments Inc., Billerica, MA). This instrument was calibrated in accordance with ASTM E1607-94 (*24*) using a set of transfer standard dosimeters (Physical Laboratory, Middlesex, UK).

Measurement of Volatile Compounds. After irradiation, fruit were transported back to the USDA ARS laboratory in Wenatchee, WA. One tray of fruit from each treatment was used for analysis of volatile compounds. There were four replicates (5 apples each) per treatment. The fruit were stored at 20 °C after irradiation and production of ethylene (*25*) and other volatile compounds (*26*) were measured on the day of irradiation, and 1, 3, 7, 14, and 21 days after irradiation. Measurement on the day of irradiation was performed on nonirradiated controls and nonirradiated MCP-treated fruit only. Fruit were placed into 4-L glass jars sealed with Teflon lids with two gas ports. Purified compressed air flowed at 6 $L \cdot h^{-1}$ through the jars and gas samples were collected from each jar outlet. For ethylene analysis, a 0.5-mL gas sample was collected using a 1-mL tuberculin syringe and analyzed using gas chromatography as described previously (*20*). Volatile compounds in the outlet gas were adsorbed onto 50 mg of 30−50 mesh Tenax TA (Alltech Associates, Deerfield, IL) packed in glass tubing (17.5 cm × 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 × 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. In total, 48 volatile compounds were monitored, including esters, alcohols, aldehydes, acids, and ketones. Esters were grouped according to the alcohol moiety: methyl-, ethyl-, propyl-, 2-methylpropyl-, butyl-, 2-methylbutyl-, pentyl- and hexyl-.

Statistical Analysis. The data were subjected to analysis of variance using SAS release 6.12 (SAS Institute, Cary, NC). Treatment differences were identified using Fisher's least significant difference (LSD) and unless indicated, only significant differences ($P < 0.05$) are discussed. To simplify the figures, mean standard deviations are indicated. Differences between means that exceed the mean standard deviations were always significant when analyzed using the LSD procedure.

RESULTS

Mean internal ethylene concentration at harvest was 5.28 $\mu L \cdot L^{-1}$ (ranging from 0.04 to 43.4 $\mu L \cdot L^{-1}$), indicating the onset of the climacteric had occurred in some fruit. Both MCP-treatment and irradiation reduced ethylene production compared to controls during the 21-day period at 20 °C (Figure 1 A and B). Irradiation reduced ethylene production by non-MCP-treated fruit in a dose-dependent manner. Irradiation of MCP-treated fruit resulted in higher ethylene production 1 day after irradiation, but no treatment effects were evident thereafter.

Treatment with MCP inhibited production of volatile compounds (Figure 1C and D). Irradiation reduced production of total volatile compounds by non-MCP-treated fruit in a dose dependent manner, but did not further reduce production by MCP-treated fruit. The inhibition of volatile production induced by a 1.32 kGy irradiation or treatment with 0.5 $\mu L \cdot L^{-1}$ MCP was similar. Volatile production by apples irradiated with 0.44 kGy began to increase between 3 and 7 days after irradiation and was similar to that of control fruit after 21 days. No unique volatile compounds were detected from MCP-treated, irradiated, or MCP-treated then irradiated fruit.

Ester production by nonirradiated control fruit increased to a maximum 7 days after the irradiation treatment. Treatment with MCP delayed the increase in ester production by 14 days and reduced the maximum rate attained over the 21-day period at 20 °C. Irradiation inhibited production of esters by both MCP-treated and control fruit (Figure 2 A and B). The degree of inhibition increased with increased irradiation dose. Ester production by MCP-treated and non-MCP-treated control fruit irradiated with 1.32 kGy were 24% and 7%, respectively, of that of nonirradiated control fruit after 21 days at 20 °C.

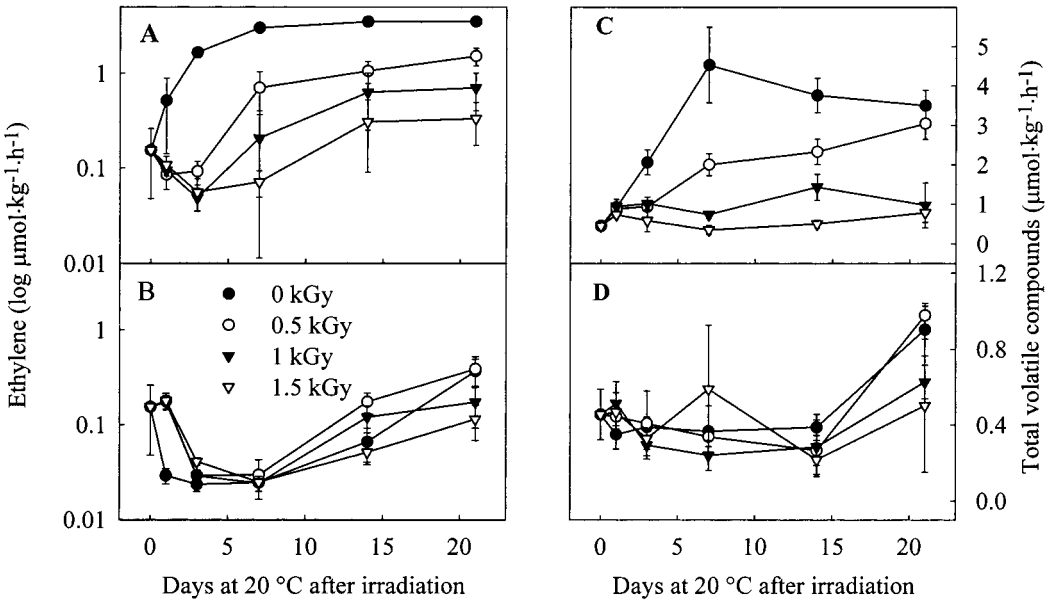

Figure 1. Production of ethylene (A, B) and total volatile compounds (C, D) of Gala apples during ripening. The fruit treated with (B, D) and without (A, C) 0.5 $\mu L \cdot L^{-1}$ MCP were exposed to 0, 0.44, 0.88, and 1.32 kGy gamma irradiation and kept at 20 °C. Vertical bars represent standard deviation.

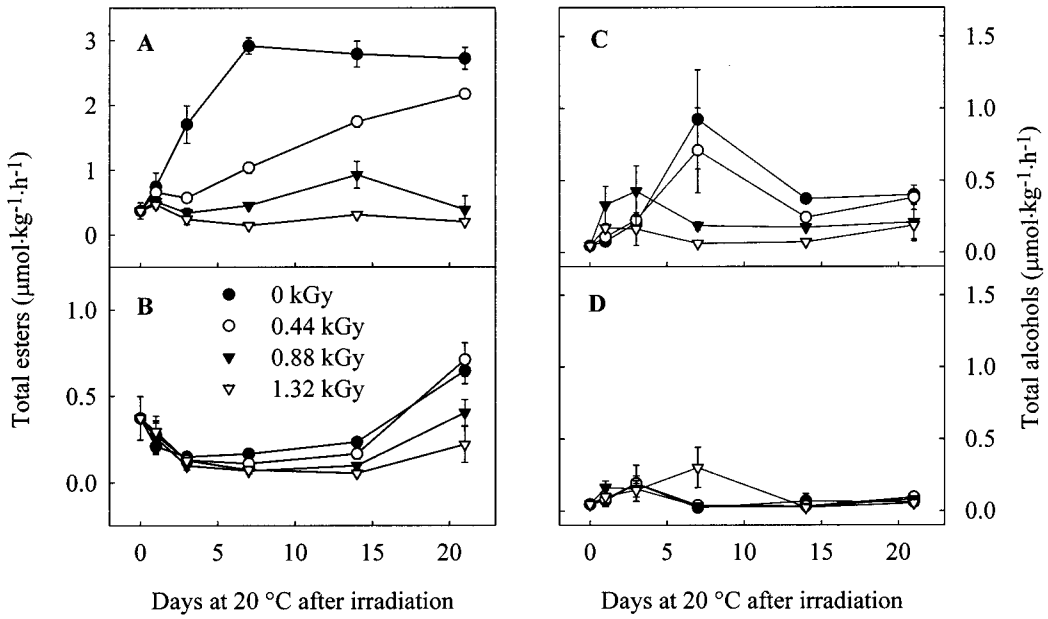

Figure 2. Production of total esters (A, B) and total alcohols (C, D) of Gala apples during ripening. The fruit treated with (B, D) and without (A, C) 0.5 $\mu L \cdot L^{-1}$ MCP were exposed to 0, 0.44, 0.88, and 1.32 kGy gamma irradiation and kept at 20 °C. Vertical bars represent standard deviation.

Production of alcohols by control fruit reached a peak on day 7 (Figure 2 C and D). MCP treatment or irradiation at doses of 0.88 and 1.32 kGy eliminated most of this increase. Alcohol production by non-MCP-treated fruit irradiated at 0.88 kGy was higher than that of nonirradiated controls 1 and 3 days after irradiation, but lower on days 7, 14, and 21. Non-MCP-treated fruit irradiated at 1.32 kGy also had lower alcohol production on days 7, 14, and 21 compared to that of the nonirradiated controls. Irradiation at 1.32 kGy resulted in increased alcohol production by MCP-treated fruit 7 days after irradiation; all other irradiation treatments results were similar to those of controls.

MCP treatment generally inhibited production of volatile aldehydes and acetic acid throughout the 21-day period at 20 °C, whereas irradiation reduced the production of aldehydes and acetic acid by control fruit only at day 7 when maximum productions were observed on nonirradiated control fruit (data not shown). MCP treatment or irradiation inhibited production of methyl esters (data not shown).

Irradiation with 0.88 kGy promoted production of ethanol by non-MCP-treated fruit 7 days after irradiation (Figure 3A). Irradiation at other doses and 0.88 kGy at other times after treatment did not impact ethanol production. Ethanol production was not altered following MCP treatment (Figure 3B). MCP treatment resulted in decreased production of ethyl esters (ethyl acetate, ethyl propanoate, ethyl butyrate, ethyl 2-methyl butyrate, ethyl pentanoate, ethyl hexanoate, and ethyl octanoate) during the entire 21-day period at 20 °C (Figure 3, C and D). Irradiation with 0.88 or 1.32 kGy

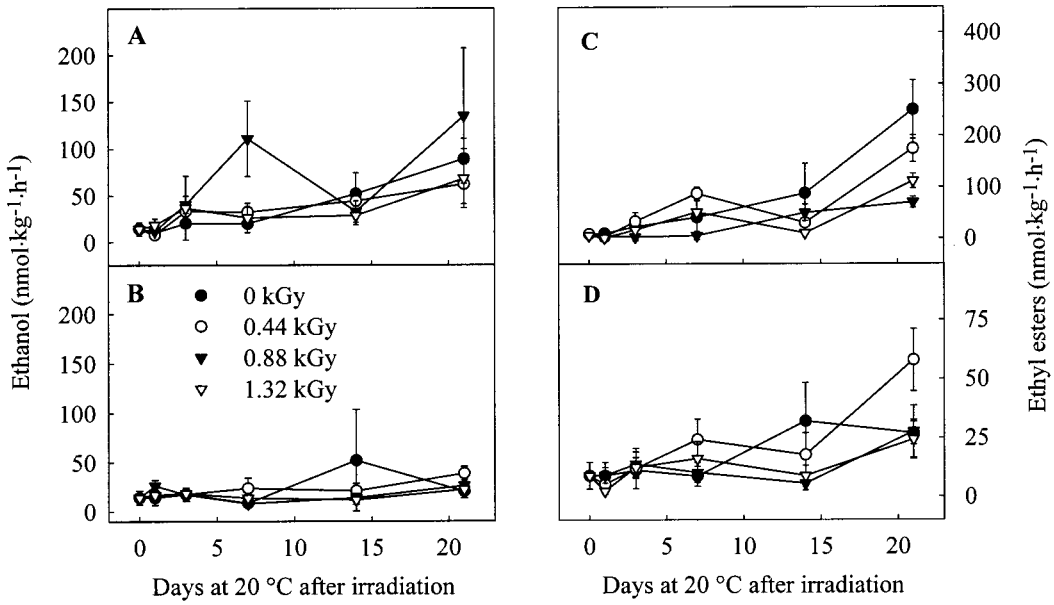

Figure 3. Production of ethanol (A, B) and ethyl esters (C, D) of Gala apples during ripening. The fruit treated with (B, D) and without (A, C) 0.5 $\mu L \cdot L^{-1}$ MCP were exposed to 0, 0.44, 0.88, and 1.32 kGy gamma irradiation and kept at 20 °C. Vertical bars represent standard deviation.

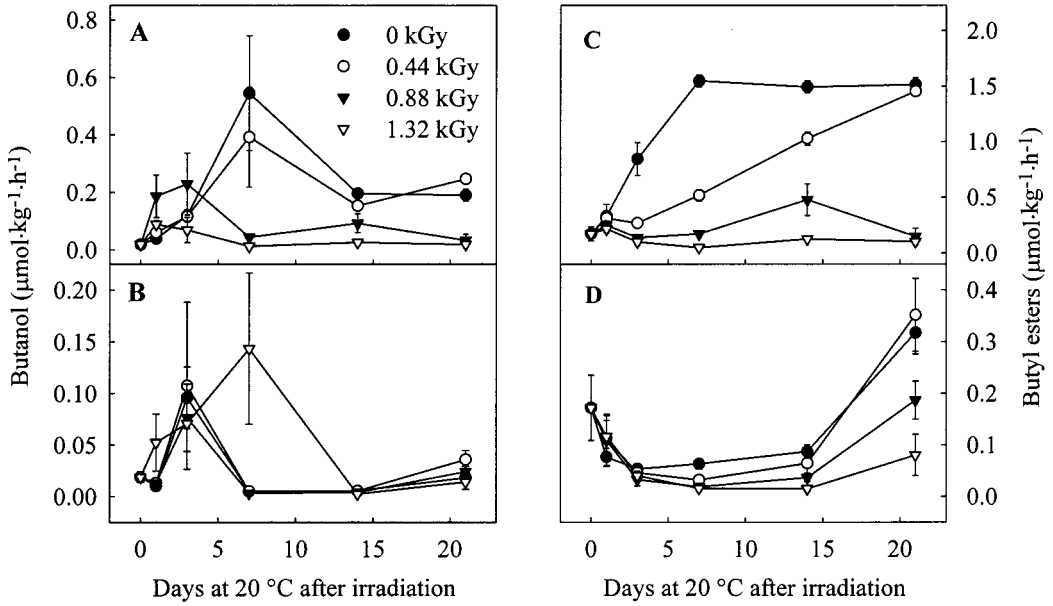

Figure 4. Production of butanol (A, B) and butyl esters (C, D) of Gala apples during ripening. The fruit treated with (B, D) and without (A, C) 0.5 $\mu L \cdot L^{-1}$ MCP were exposed to 0, 0.44, 0.88, and 1.32 kGy gamma irradiation and kept at 20 °C. Vertical bars represent standard deviation.

resulted in reduced production of ethyl esters by non-MCP-treated fruit 21 days after irradiation. Apples treated with MCP then irradiated at 0.44 kGy had higher ethyl ester production compared to those receiving other irradiation doses 21 days after treatment.

Butanol production by nonirradiated controls and control fruit irradiated with 0.44 kGy reached a peak after 7 days at 20 °C (Figure 4A). Peak butanol production by apples irradiated at 0.88 kGy occurred after 3 days, however, production was lower compared to that of controls or fruit receiving 0.44 kGy (Figure 4A). Peak butanol production also occurred earlier in MCP-treated fruit with the exception of fruit irradiated with 1.32 kGy (Figure 4B). Butanol production was reduced in all MCP-treated fruit compared to that of controls. Production of butyl esters (butyl acetate, butyl propanoate, butyl butyrate, butyl 2-methyl butyrate, and butyl hexanoate) by control fruit increased from day 0 to day 7 and then plateaued (Figure 4C). MCP treatment delayed the increase in butyl ester production (Figure 4D). Irradiation reduced butyl ester production by both MCP-treated- and nontreated- fruit. The reduction in production increased with radiation dose. Butyl ester production by non-MCP-treated fruit irradiated with 0.44 kGy began to increase between 3 and 7 days after irradiation and was similar to that of controls at 21 days.

Irradiation treatments resulted in reduced hexanol production by non-MCP-treated fruit between 3 and 14 days after irradiation. Hexanol production decreased as irradiation dose increased (Figure 5A). Hexanol production by controls and fruit irradiated at 0.44 kGy was

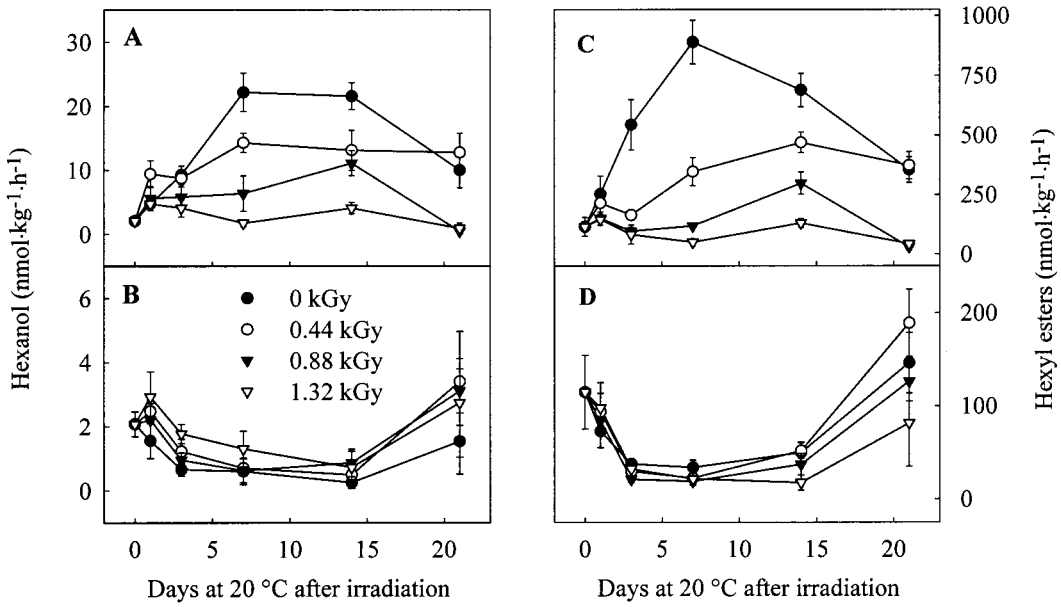

Figure 5. Production of hexanol (A, B) and hexyl esters (C, D) of Gala apples during ripening. The fruit treated with (B, D) and without (A, C) 0.5 $\mu L \cdot L^{-1}$ MCP were exposed to 0, 0.44, 0.88, and 1.32 kGy gamma irradiation and kept at 20 °C. Vertical bars represent standard deviation.

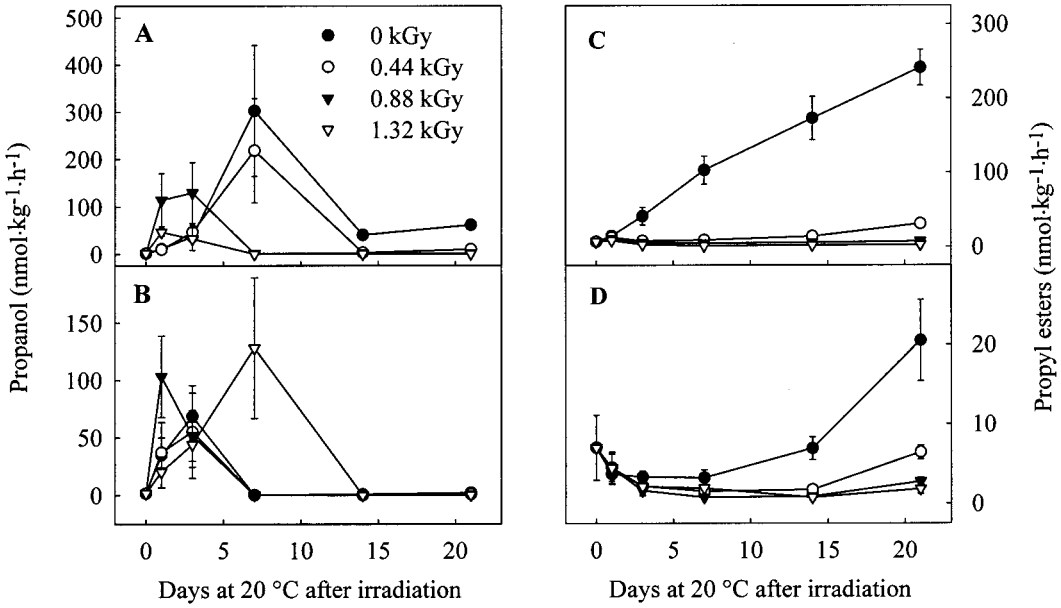

Figure 6. Production of propanol (A, B) and propyl esters (C, D) of Gala apples during ripening. The fruit treated with (B, D) and without (A, C) 0.5 $\mu L \cdot L^{-1}$ MCP were exposed to 0, 0.44, 0.88, and 1.32 kGy gamma irradiation and kept at 20 °C. Vertical bars represent standard deviation.

similar 21 days after irradiation. MCP treatment resulted in reduced hexanol production relative to control fruit, but irradiation had no additional effect on hexanol production by MCP-treated fruit (Figure 5B). Changes in production of hexyl esters (hexyl acetate, hexyl propanoate, hexyl butyrate, and hexyl 2-methylbutyrate) induced by MCP-treatment and/or irradiation were similar to those for hexanol production (Figure 5C and D).

Patterns of propanol production were similar to those of butanol (compare Figure 6A and B to Figure 4A and B). Production of propyl esters (propyl acetate, propyl propanoate, and propyl hexanoate) by control fruit increased linearly during the 21-day period at 20 °C. Both MCP-treatment and irradiation reduced propyl ester production (Figure 6C and D). Irradiation of MCP-treated fruit further reduced propyl ester production (Figure 6D).

Production of pentanol by control fruit peaked after 7 days at 20 °C. Treatment with MCP or irradiation prevented the increase in pentanol production (Figure 7A). Irradiation of MCP-treated fruit had no additional impact on pentanol production (Figure 7B). The pattern of pentyl ester production (pentyl acetate and pentyl butyrate) following MCP treatment and/or irradiation was similar to the production profile of hexyl and butyl esters (Figure 7C and D).

Production of 2-methyl-1-propanol decreased following MCP treatment (Figure 8A and B). Irradiation at 1.32 kGy resulted in decreased 2-methyl-1-propanol production by non-MCP-treated fruit 21 days after irradiation (Figure 8A and B). Irradiation at doses below 1.32 kGy

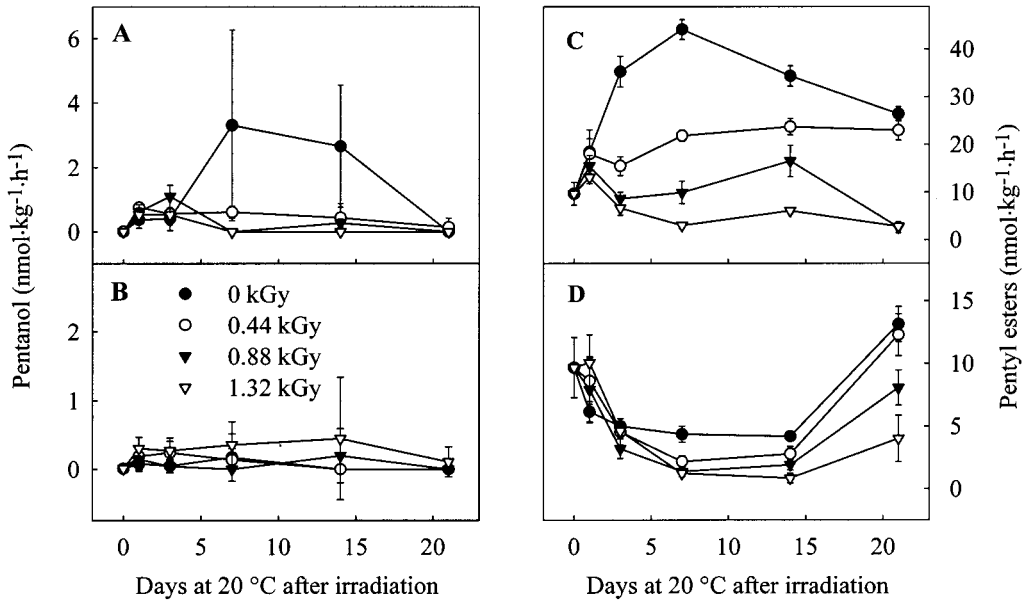

Figure 7. Production of pentanol (A, B) and pentyl esters (C, D) of Gala apples during ripening. The fruit treated with (B, D) and without (A, C) 0.5 $\mu L \cdot L^{-1}$ MCP were exposed to 0, 0.44, 0.88, and 1.32 kGy gamma irradiation and kept at 20 °C. Vertical bars represent standard deviation.

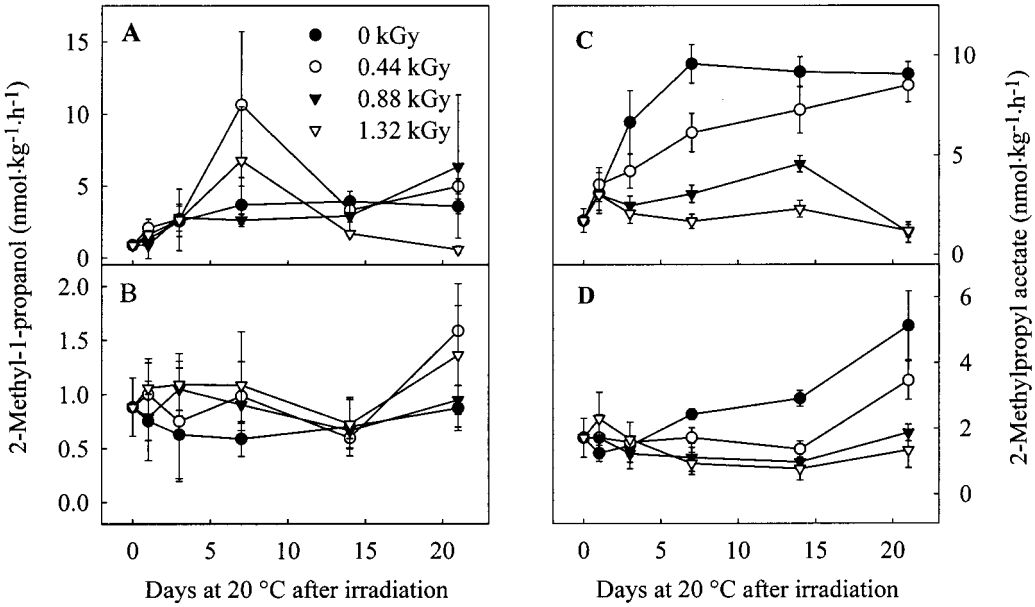

Figure 8. Production of 2-methylpropanol (A, B) and 2-methylpropyl acetate (C, D) of Gala apples during ripening. The fruit treated with (B, D) and without (A, C) 0.5 $\mu L \cdot L^{-1}$ MCP were exposed to 0, 0.44, 0.88, and 1.32 kGy gamma irradiation and kept at 20 °C. Vertical bars represent standard deviation.

did not impact 2-methyl-1-propanol production. Production of 2-methylpropyl acetate (Figure 8C and D) decreased after MCP treatment, and irradiation treatments inhibited production of 2-methylpropyl acetate in both MCP-treated and nontreated fruit. The amount of inhibition increased with radiation dose. Production of 2-methylpropyl acetate by fruit irradiated with 0.44 kGy increased to a rate similar to that of control fruit by 21 days after the irradiation treatment.

A continuous increase in 2-methyl-1-butanol production by control fruit was observed during the 21-day period at 20 °C, and the increase was prevented by treatment with MCP or the 0.88 kGy and 1.32 kGy irradiation treatments (Figure 9A). Irradiation of MCP-treated fruit did not impact production of this alcohol (Figure 9B). Production of 2-methylbutyl esters (2-methylbutyl acetate and 2-methylbutyl 2-methyl butyrate) was also inhibited after MCP treatment (Figure 9C and D). Irradiation inhibited production of 2-methylbutyl esters by both MCP- and non-MCP-treated fruit in a dose-dependent fashion. Irradiation at doses of 0.88 or 1.32 kGy induced effects similar to those of MCP on production of 2-methylbutyl esters. Production of 2-methylbutyl esters by MCP-treated fruit and fruit irradiated at 0.44 kGy increased between days 14 and 21 at 20 °C.

DISCUSSION

The majority, qualitatively and quantitatively, of volatile compounds produced by ripening apple fruit are esters, alcohols, aldehydes, and ketones (*17*). Apple fruit

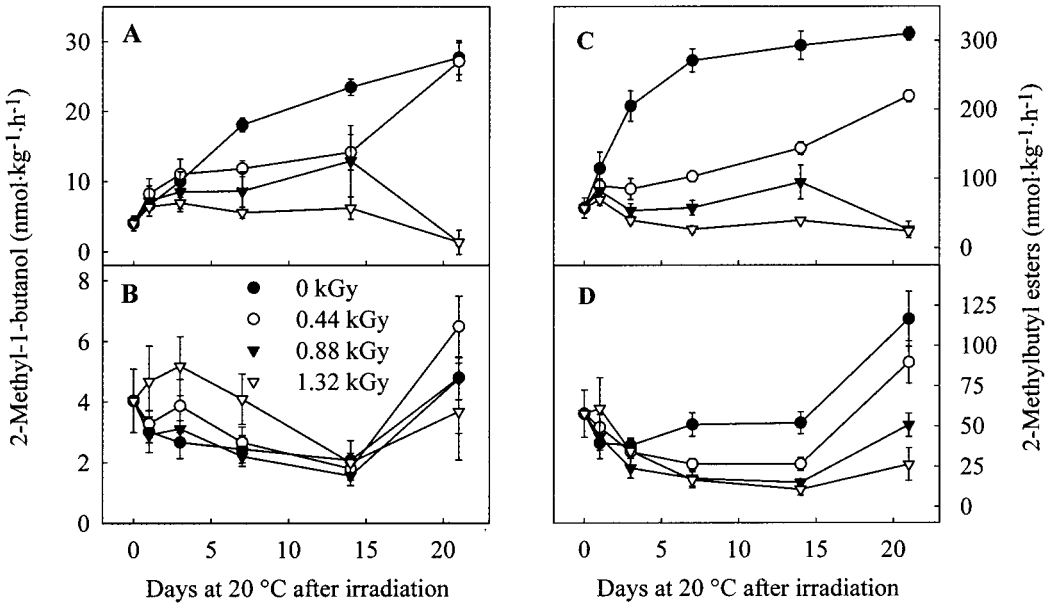

Figure 9. Production of 2-methylbutanol (A, B) and 2-methylbutyl esters (C, D) of Gala apples during ripening. The fruit treated with (B, D) and without (A, C) 0.5 $\mu L \cdot L^{-1}$ MCP were exposed to 0, 0.44, 0.88, and 1.32 kGy gamma irradiation and kept at 20 °C. Vertical bars represent standard deviation.

ester production is catalyzed enzymatically by coupling the respective carboxylic acid and alcohol (*27*−*30*), therefore, if the amount of either of these substrates decreases, ester production can be limited. Apples stored for long periods in low O_2 and/or high CO_2 have reduced production of esters due in part to a limitation in alcohol availability (*28* and *31*). Although butyl esters are the major volatile compounds of Gala apples (*32* and *33*), the profiles of butyl alcohol and butyl ester production were different (Figure 4). The irradiation treatments conducted in this study typically impacted production of esters more than alcohols indicating that irradiation may disrupt the capacity to produce esters. Properties of the enzyme(s) responsible for ester synthesis, such as alcohol acetyltransferase (*30*), may be impacted. Tobback et al. (*9*) indicated impacts of irradiation at 0.5, 2, and 5 kGy inhibited production of most fruit volatile compounds but stimulated the production of ethanol, methanol, and acetaldehyde. Methanol and acetaldehyde could not be detected by the method we used. We did observe that 0.88 kGy promoted production of ethanol by non-MCP-treated fruit 7 days after irradiation, but other doses had no effect on ethanol production.

Although irradiation has a number of potential commercial benefits, including insect disinfestation, disease control, and reduction of some physiological disorders, it promotes fruit softening (*34* and *38*) and impacts fruit volatile production. Although these impacts of irradiation on aspects of fruit quality have been established using analytical techniques, whether irradiation alters consumer acceptance of apple fruit is as yet unknown.

Irradiation at 0.88 or 1.32 kGy resulted in impaired ester production for Gala apples stored 21 days at 20 °C following irradiation treatment. The production of many esters by non-MCP-treated fruit exposed to 0.88 and 1.32 kGy was the lowest 21 days after irradiation, indicating the reduction in ester production in these fruit may be permanent. Fruit irradiated at a lower dose (0.44 kGy) did recover the ability to produce esters and other volatile compounds. The rate of ester production by fruit irradiated at 0.44 kGy was similar to that of controls 21 days after irradiation. Although minimum doses of 0.05−0.25 kGy are required for disinfestation of most arthropod insects, much fruit may receive more than 0.3 kGy when applied on a commercial scale (*4*). Doses above 1.0 kGy are needed for disease control in apple fruit (*1* and *35*). Our results indicate the use of irradiation as an insect quarantine control treatment is unlikely to permanently affect volatile production, but irradiation at levels required for disease control is likely to impair production of many volatile compounds.

Sensitivity of fruit to irradiation varies among the different groups of volatile compounds. Production of esters was more sensitive to irradiation than alcohols, aldehydes, and acetic acid. Irradiation inhibited production of all esters by non-MCP-treated fruit. MCP treatment alone resulted in a low rate of esters production, and irradiation after MCP treatment further reduced production of most esters somewhat. Among the alcohols, irradiation consistently inhibited production of hexanol and 2-methylbutanol. Among the esters produced by Gala apples, methyl and propyl esters were the most influenced by irradiation, similar to the effect of MCP (*20*). Production of methyl and propyl esters by control fruit increased continuously during the 21 days at 20 °C and this increase was prevented by irradiation. Production of the major quantitative esters including butyl and hexyl esters was also very sensitive to irradiation.

Both MCP and irradiation inhibited production of ethylene and other volatile compounds. The effects of irradiation cannot be easily distinguished from those of MCP. The results appear to indicate irradiation does impact ethylene action in apple fruit. First, the effects of irradiation are very similar to those induced by MCP, an ethylene action inhibitor. Second, MCP influenced the response of fruit to radiation dose. Irradiation inhibited production of hexanol (Figure 5A and B) and 2-methylbutanol (Figure 9A and B) by non-MCP-treated fruit, but had little effect on the production by MCP-treated fruit. These results indicate that at least a portion of the responses induced by irradiation may require ethylene action. Exposure of Bartlett pears to ethylene following irradiation does not reverse the

inhibition of ripening by irradiation (*36*), indicating that irradiation may inhibit ethylene action.

Our results indicate that irradiation inhibited ethylene production by non-MCP-treated fruit at all doses used in this study, and ethylene production decreased as irradiation dose increased. Irradiation inhibits 1-aminocyclopropane-1-carboxylic acid oxidase (*37*), the key enzyme in ethylene biosynthesis pathway. A previous report showed that doses of 0.6 kGy or higher decrease ethylene production, whereas doses less than 0.5 kGy stimulate ethylene production by apple fruit (*21*). Lack of stimulation of ethylene production following irradiation at 0.44 kGy may be due to differences in how cultivars respond to irradiation, or treatment at different physiological stages of development. Ethylene production was higher in MCP-treated fruit 1 day after irradiation but no consistent differences were observed for the remainder of the 21-day period. The initial stimulation of ethylene synthesis by irradiation may be the result of activated translation of preexisting mRNAs for 1-aminocyclopropane-1-carboxylic acid oxidase (*37*).

Although irradiated fruit had lower ethylene production compared to that of the controls, ethylene production was still detectable. Ethylene production above a minimal amount may be irrelevant in terms of its stimulation of some ripening-related processes (*19*). A high amount of ethylene production and continuous ethylene action is, however, required for a high rate of volatile production for many compounds (*20*), at least in the early phase of ripening (*19*). The low production rate of many esters and alcohols by irradiated fruit may have resulted from the low rate of ethylene production, and reflected a limitation in precursors associated with the reduced rates of respiration (data not shown).

In summary, production of volatile esters and alcohols by apple was reduced following irradiation treatments. The volatile production rate decreased as irradiation dose increased. Ester production appears to be more sensitive to irradiation than alcohol production, indicating reduced synthesis of esters may not be limited by alcohol availability in irradiated fruit. There is also variation in sensitivity to irradiation among different groups of volatile esters. Fruit irradiated at 0.44 kGy recovered the capacity to generate volatile esters during 21 days at 20 °C, but ester production of fruit irradiated at 0.88 or 1.32 kGy appeared to be permanently impaired.

ACKNOWLEDGMENT

We thank Donald Thayer for advice on dosimetry, Michael Brown for irradiating the fruit and suggestion on dosimetry, David Buchanan and Janie Gausman for technical assistance, and Biotechnolgy for Horticulture, Inc., for providing Ethylbloc.

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Received for review July 19, 2000. Revised manuscript received October 17, 2000. Accepted October 30, 2000.

JF0008994