Effects of irradiation on respiration and ethylene production of apple slices
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Abstract: Respiration and ethylene production rates of irradiated apple slices from four apple cultivars were measured for 72h. Doses less than 1.2kGy had no effect on rates of CO₂ production and O₂ consumption, and irradiation at doses between 1.2 and 2.4kGy had minimal effect for all cultivars. Respiratory response of ‘Idared’, ‘Law Rome’ and ‘Empire’ slices to irradiation was curvilinear, with maximum respiration occurring in the 3±6kGy dose range. Response of ‘Delicious’ slices was linear over the irradiation dose range (0±11kGy) studied. Respiratory quotient increased with irradiation dose. The degree of maturity of the slices affected respiratory responses. Irradiation reduced ethylene production of all slices. These results suggest that irradiation doses of up to 2.4kGy can be used with minimum effect on the respiratory physiology of tissues.

INTRODUCTION
Demand for minimally processed fruits and vegetables has increased owing to consumer preferences for fresh and convenient food products.1 Apple is the third most valuable fruit grown in the United States, with a value of $1.7 billion in 1996,2 and while more than 50% of the apple fruit is sold fresh,3 new processes are needed to add value to the fruit. Shelf-life of minimally processed apples can be extended through combinations of chemical treatments with modified atmosphere packaging,4,5 but further shelf-life extension and without microbiological contamination is desirable. Recently, irradiation has been proposed for a variety of fresh and processed foods involving different treatment conditions and irradiation doses, especially as a control for emergence of food-borne illnesses.6,7 Response of fresh fruit respiration to irradiation depends on cultivar, maturity and irradiation dose levels.8 Irradiation resulted in stimulation of respiration rate of apples, and generally tissues from more mature fruit were less sensitive to irradiation.8,9 Romani et al10 observed a divergent respiratory response of ‘Bartlett’ pears to irradiation dose, with higher levels of irradiation (6–10kGy) resulting in a reduced respiratory activity compared with that of control fruits or those irradiated at lower levels (2–2.5kGy).

METHODS
Apple harvest
Approximately 50kg of apples were harvested from mature trees of ‘Delicious’, ‘Empire’, ‘Idared’ and ‘Rome’ cultivars growing at Cornell Orchards, Ithaca on 23 September, 30 September, 7 October and 12 October 1998 respectively. Fruit maturity of 10-fruit samples was assessed by measuring the internal ethylene concentration (IEC) on 1ml samples of internal gas from the core cavity of each fruit by a...
gas chromatograph (Hewlett Packard 5890, Series II, Wilmington, DE). The apples were used for experiments on the day following harvest or after 7 months of storage in CA (2% O2 and 2% CO2 at 0°C for ‘Delicious’ or at 1.5°C for ‘Empire’). IEC of stored fruit was determined as above.

Preparation and irradiation
Six separate experiments were carried out with pre-climacteric ‘Delicious’, ‘Empire’ ‘Idared’ and ‘Law Rome’ apples and post-climacteric ‘Delicious’ and ‘Empire’ apples as judged by IEC. Apples were peeled, cored and sliced into eight wedges using a hand peeler and slicer, and immersed in cold tap water. Slices were then vacuum-packed in single layers using high-barrier polyethylene pastic bags with a Multivac packaging machine (Koch, Kansas City, MO). Packages were placed in an upright position in metal racks inside insulated styrofoam containers containing ice-water, and irradiated using a point source consisting of 13 60Co pencils, each 19 cm long, with a total power of 10000Ci at The Ward Laboratory of Nuclear Sciences, Cornell University. The temperature of the samples during irradiation was kept at 0±3°C. Each container was placed at an appropriate distance from the gamma source to obtain the desired dose levels. For each dose level, triplicate samples in separate bags were irradiated for 5h. Control samples were treated identically but kept outside the irradiation chamber during the treatment.

Respiration rate and ethylene production measurements
Following irradiation, the bags were opened, and weighed slices (150g) were placed in respiratory jars (500ml). Jars were kept open at 5°C and 95% RH in a high-humidity cold store. After equilibration of the apple slice temperatures, respiration rate and ethylene production were measured using a closed system.14 Changes in CO2 and O2 concentration in the headspace of the jars over 3h were measured with a gas chromatograph (Fisher Gas Partitioner, Model 1200, Fisher Scientific, Springfield, NJ) equipped with a thermal conductivity detector. Column and injector temperatures were 90 and 135°C respectively. Head-space ethylene concentration was measured with a gas chromatograph (Hewlett Packard 5710A) equipped with a flame ionisation detector. Column, detector and injector temperatures were 140, 300 and 250°C respectively. Respiration and ethylene production were measured after 4, 24 and 72h following irradiation. Between sampling times the slices were kept in open jars.

Organic acid analysis
For determination of organic acids of post-climacteric ‘Delicious’ and ‘Empire’ apple slices, jar contents were

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**Figure 1.** CO2 production rates of ‘Law Rome’ and ‘Idared’ apple slices during 72h storage at 5°C as affected by irradiation dose.

**Figure 2.** O2 consumption rates of ‘Law Rome’ and ‘Idared’ apple slices during 72h storage at 5°C as affected by irradiation dose.
frozen in liquid N₂ and stored at −20°C until used. Frozen slices in liquid N₂ were ground to a fine powder using a coffee grinder. Extraction and quantification of organic acids from 1 g of powder by gas chromatograph were performed by the method of Mattick et al. 15

**Statistical analysis**

Data were analysed by the General Linear Model procedure to determine effects of irradiation dose and post-irradiation storage period for each cultivar using Minitab, release 12 (Minitab Inc, State College, PA). Tukey's multiple comparison at 95% level was performed to compare individual doses and post-irradiation periods. To determine the effects of cultivar and post-irradiation period on response of slices to irradiation, the CO₂ production and O₂ consumption rate data (at 4 and 72h post-irradiation periods) were analysed by a regression procedure. 16 Since the main trends for all the cultivars except ‘Delicious’ were curvilinear, a series of quadratic models that indicate all possible combinations of cultivar and post-irradiation period effects were fitted and the best model was selected using F-tests. 16 The effect of ripeness on the response of the tissues to irradiation was assessed similarly by analysing the data obtained for pre- and post-climacteric ‘Delicious’ and ‘Empire’ apple slices. Since ‘Delicious’ slices did not have a curvilinear response within 0–11 kGy doses, series of linear models were fitted and the best model was selected using F-tests. Quadratic models were fitted to respiration data of pre- and post-climacteric ‘Empire’ slices and the best model was selected to determine the effect of maturity stage on response to irradiation.

**RESULTS AND DISCUSSION**

Rates of CO₂ production and O₂ consumption of pre-climacteric ‘Idared’ and ‘Rome’ apple slices increased with irradiation dose above 1.2 kGy (P < 0.01). Respiration rates were higher within the 3–5 kGy dose range but lower at higher doses (Figs 1 and 2). Moreover, higher respiration rates were observed at 4h post-irradiation than at 24 and 72h (P < 0.01), presumably owing to wound-induced responses of tissues. Difference among doses decreased over time. High dose levels (7.5 and 11 kGy) generally resulted in lower respiration rates than control (0 kGy) at 24 and 72h.

Irradiation of pre-climacteric ‘Empire’ slices with doses from 0 to 11 kGy also stimulated both CO₂ production and O₂ consumption (Figs 3 and 4). Both

![Figure 3. CO₂ production rates of pre- and post-climacteric ‘Empire’ and ‘Delicious’ apple slices during 72h storage at 5°C as affected by irradiation dose.](image-url)
dose level and post-irradiation period affected respiration rate ($P < 0.01$). CO$_2$ production rate was higher within 4–7 kGy doses compared to control and other doses (Fig 3). Therefore pre-climacteric apple slices of ‘Idared’, ‘Law Rome’ and ‘Empire’ behaved similarly, with highest respiration rates within the 3–7 kGy dose range.

In contrast, the respiration rate of ‘Delicious’ slices (both rates of CO$_2$ production and O$_2$ consumption) increased with increasing dose levels without any apparent maximum within the dose range studied. Respiration rate of ‘Delicious’ slices increased with increasing dose levels above 2.4 kGy at each post-irradiation period (Figs 3 and 4).

Differences among responses of slices to irradiation due to cultivar and post-irradiation period were obtained by regression analysis of the data. The following model best fitted for CO$_2$ production and O$_2$ consumption data of the four pre-climacteric cultivars (Tables 1 and 2):

$$\text{Rate} = \beta_{0ij} + \beta_{1ij} \times \text{Dose} + \beta_{2ij} \times \text{Dose}^2 + \epsilon_{ij}$$

where $j$ is the post-irradiation period (4, 72h), $i$

### Table 1. Regression equations fitted for rate of CO$_2$ production of pre-climacteric apple slices. Each equation was extracted from the best-fitted model with an $R^2$ of 0.946

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Time (h)</th>
<th>Regression equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idared</td>
<td>4</td>
<td>$R_{CO2} = 3.970 + 0.605 D - 0.058 D^2$</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>$R_{CO2} = 1.320 + 0.500 D - 0.049 D^2$</td>
</tr>
<tr>
<td>Delicious</td>
<td>4</td>
<td>$R_{CO2} = 2.080 + 0.634 D - 0.027 D^2$</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>$R_{CO2} = 1.210 + 0.042 D + 0.004 D^2$</td>
</tr>
<tr>
<td>Empire</td>
<td>4</td>
<td>$R_{CO2} = 3.215 + 0.657 D - 0.048 D^2$</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>$R_{CO2} = 1.318 + 0.421 D - 0.043 D^2$</td>
</tr>
<tr>
<td>Law Rome</td>
<td>4</td>
<td>$R_{CO2} = 3.486 + 0.280 D - 0.018 D^2$</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>$R_{CO2} = 1.297 + 0.247 D - 0.024 D^2$</td>
</tr>
</tbody>
</table>

$R_{CO2}$, CO$_2$ production rate; D, irradiation dose (kGy); 4 and 72h, post-irradiation, period.

### Table 2. Regression equations fitted for rate of O$_2$ consumption of pre-climacteric apple slices. Each equation was extracted from the best-fitted model with an $R^2$ of 0.964

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Time (h)</th>
<th>Regression equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idared</td>
<td>4</td>
<td>$R_{O2} = 4.210 + 0.313 D - 0.040 D^2$</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>$R_{O2} = 2.175 + 0.358 D - 0.047 D^2$</td>
</tr>
<tr>
<td>Delicious</td>
<td>4</td>
<td>$R_{O2} = 5.496 + 0.153 D + 0.008 D^2$</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>$R_{O2} = 1.993 - 0.045 D + 0.011 D^2$</td>
</tr>
<tr>
<td>Empire</td>
<td>4</td>
<td>$R_{O2} = 3.600 + 0.350 D - 0.040 D^2$</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>$R_{O2} = 2.440 + 0.183 D - 0.030 D^2$</td>
</tr>
<tr>
<td>Law Rome</td>
<td>4</td>
<td>$R_{O2} = 4.000 + 0.070 D - 0.012 D^2$</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>$R_{O2} = 2.016 + 0.263 D - 0.032 D^2$</td>
</tr>
</tbody>
</table>

$R_{O2}$, O$_2$ consumption rate; D, irradiation dose (kGy); 4 and 72h, post-irradiation, period.

![Figure 4. O$_2$ consumption rates of pre- and post-climacteric ‘Empire’ and ‘Delicious’ apple slices during 72h storage at 5°C as affected by irradiation dose.](image-url)
denotes the cultivar (1, Idared; 2, Delicious; 3, Empire; 4, Law Rome), Dose is the irradiation dose in kGy and Rate is the rate of CO₂ production or O₂ consumption in ml kg⁻¹ h⁻¹. A sample graph illustrating the quadratic curves with the fitted equations and individual $R^2$ for both CO₂ production and O₂ consumption of pre-climacteric 'Empire' slices is shown in Fig 5. The above model indicates effect of cultivar on respiratory response of apple slices to irradiation. It also indicates that post-irradiation period not only affected the magnitude of respiration rate but also influenced the rate of change of respiration by irradiation dose.

The mechanism of the increase in respiration by irradiation is not well known. Massey and Bourke¹⁷ found that irradiation stimulated catabolism of acetate to CO₂ in carrot tissues, and postulated that this explains the increased CO₂ evolution by irradiation. In contrast to our observations on apple slices, irradiation has been reported to inhibit respiration rate of fresh produce. For instance, Chervin et al¹¹ found that irradiation at 2kGy dose resulted in about 50% reduction in respiration rate of grated carrots. Moreover, an inhibitory effect of irradiation at 3kGy on respiration rate was also reported for intact tomatoes.¹⁸ Thus response of plant tissues to irradiation depends on commodity, highlighting the need to define the response of each produce item separately.

Physiological age also influenced the responses of the tissues to irradiation. Responses of pre- and post-climacteric ‘Delicious’ tissues to irradiation differed (Figs 3 and 4). The best-fitted linear model for CO₂ production and O₂ consumption was

$$\text{Rate} = \beta_{0ij} + \beta_{1ij} \cdot \text{Dose} + \epsilon_{ij}$$

where $i$ denotes the ripeness (1, pre-climacteric; 2, post-climacteric) and $j$ is the post-irradiation period (Table 3). The model indicates effects of both stage of maturity and post-irradiation time on respiration rate. The slopes of the regression lines for both rates of CO₂ production and O₂ consumption were greater for pre-climacteric slices than post-climacteric slices (Table 3). Therefore respiration rate of pre-climacteric ‘Delicious’ slices increased faster than that of post-climacteric slices. A similar increase in respiration rate of pre-climacteric compared with post-climacteric slices.

![Figure 5. Fitted curves for CO₂ production and O₂ consumption of pre-climacteric ‘Empire’ apple slices at 4h and 72h post-irradiation periods.](image)

**Figure 5. Fitted curves for CO₂ production and O₂ consumption of pre-climacteric ‘Empire’ apple slices at 4h and 72h post-irradiation periods.**

![Figure 6. Respiratory quotients of pre-climacteric ‘Delicious’ and ‘Empire’ apple slices as affected by irradiation dose.](image)

**Figure 6. Respiratory quotients of pre-climacteric ‘Delicious’ and ‘Empire’ apple slices as affected by irradiation dose.**

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Pre-climacteric</th>
<th>Post-climacteric</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4h</td>
<td>72h</td>
</tr>
<tr>
<td>Delicious</td>
<td>RCO₂</td>
<td>2.487 + 0.344 D</td>
</tr>
<tr>
<td></td>
<td>RO₂</td>
<td>5.379 + 0.237 D</td>
</tr>
<tr>
<td>Empire</td>
<td>RCO₂</td>
<td>3.215 + 0.657 D – 0.048 D²</td>
</tr>
<tr>
<td></td>
<td>RO₂</td>
<td>3.939 + 0.267 D – 0.035 D²</td>
</tr>
</tbody>
</table>

| RCO₂, CO₂ production rate; RO₂, O₂ consumption rate; D, irradiation dose (kGy); 4 and 72h, post-irradiation period. |

Table 3: Regression equations fitted to respiration data of pre- and post-climacteric ‘Delicious’ and ‘Empire’ apple slices. Each equation was extracted from the best-fitted model.
apples was reported for whole ‘McIntosh’, ‘Cortland’ and ‘Rome’ apples. In contrast to ‘Delicious’ slices, the rate of CO₂ production of post-climacteric ‘Empire’ slices was stimulated to a greater extent than that of pre-climacteric tissues (Fig 3). Coefficients of the first-order terms in the regression equations for CO₂ production were larger for post-climacteric slices than for pre-climacteric slices (Table 3). On the other hand, O₂ consumption rate of post-climacteric ‘Empire’ slices was affected less than that of pre-climacteric slices (Fig 4). Therefore the maturity stage of the slices has an effect on the response of slices to irradiation, and it may interact with cultivar.

Respiratory quotient (RQ) of ‘Empire’ and ‘Delicious’ apple slices increased with increasing irradiation dose (Fig 6), indicating that CO₂ production was stimulated to a larger extent than O₂ consumption by irradiation. The increase in RQ with irradiation dose was also observed with other cultivars and post-climacteric slices (data not shown). Change in RQ decreased as the post-irradiation period increased (data not shown). Increases in RQs have also been reported for irradiated whole citrus fruits. We investigated the possibility that higher RQ was associated with organic acid levels in tissues, because it has been reported that irradiation of certain organic acids such as formic and acetic acids results in formation of CO₂ and other organic acids, including tartaric, citric, malonic and glycolic acids. However, we determined no effect of irradiation on malic acid levels, average malic acid levels being 12 and 30 µmol g⁻¹ for ‘Delicious’ and ‘Empire’ slices respectively.

Rates of ethylene production of fresh apple slices decreased with increasing irradiation dose, the reduction being greater in ‘Empire’ slices than in ‘Delicious’ slices (Fig 7). The dose required for a 50% reduction in ethylene synthesis of ‘Empire’ slices was about 1.2 kGy, while it was about 7 kGy for ‘Delicious’ slices at 4 h. Inhibition of ethylene synthesis of grated carrots by irradiation (2 kGy) has also been reported. Inhibitory effect of irradiation on ethylene production may be beneficial, since ethylene may accelerate tissue senescence. Reduction in ethylene production by irradiation decreased with post-irradiation storage period for both ‘Delicious’ and ‘Empire’ slices. Biosynthesis of ethylene in apples starts with methionine as main substrate and requires ATP and critical enzymes such as ACC synthase and ACC oxidase. Whether the inhibitory effect of irradiation on ethylene production is due to change in the substrate concentration, enzyme activity or energy level is not known.

CONCLUSIONS
Irradiation of fresh apple slices increased the respiration rate and decreased ethylene production in a dose-dependent manner above 1.2 kGy. Stimulation of respiration rate decreased within 72 h of irradiation, and therefore, in the longer term, effects of irradiation on metabolic rates may be minimal. Employing different irradiation temperature, atmosphere and dose rate can optimise the irradiation treatment through minimising its adverse effects. Although increased respiration due to high irradiation doses would be expected to reduce storage life of slices, lower doses (up to 2.4 kGy), which had minimal effect on tissue physiology, could be used in combination with other preservation techniques to improve quality and safety of minimally processed apples. FDA approval of irradiation of fresh produce is currently limited to 1 kGy, but new petitions for higher doses will likely appear in the future owing to increased interest in use of irradiation to control food-borne illnesses. Softening of tissues upon irradiation may be a limiting factor to use of the technology, as our
preliminary data have shown that greater softening of slices occurred at doses above 0.4kGy. However, the commercial impact of such softening is not yet known. The effect of the optimal irradiation treatment along with antibrowning and anti-softening treatments and modified atmosphere packaging on organoleptic (texture, colour, flavour) and microbial quality of minimally processed apples are being studied in our laboratory.

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REFERENCES