Effects of Gamma Irradiation on the Texture of Minimally Processed Apple Slices

G. Gunes, J.H. Hotchkiss, and C.B. Watkins

ABSTRACT: Fresh-cut apples with or without calcium treatments were irradiated at doses up to 5 kGy at 0.4 and 2 kGy.h\(^{-1}\) dose rates with a Co\(^{60}\) source in 0, 21, and 100% O\(_2\) atmospheres. Firmness decreased as irradiation dose increased beyond a 0.34 kGy threshold. High dose rate initially resulted in less softening compared to low dose rate, but dose rate became insignificant upon storage. Irradiation atmosphere did not affect firmness. Softening of irradiated slices correlated positively with water-soluble pectin, and negatively with oxalate-soluble pectin content. Calcium prevented irradiation-induced softening of thin slices (3-4 mm thick), but was not effective with thicker wedges due to limited penetration.

Key words: irradiation, texture, apple slices, minimally processed, calcium

Introduction

Consumer demand for minimally processed fresh produce has been increasing due to premium product quality, convenience, and fresh-like character (Ohlsson 1994). Although great interest in minimally processed fruit products exists, little commercial success has been achieved (Gorny and Kader 1996). Several preservation methods including antioxidant treatment, modified atmosphere packaging, refrigeration, chlorination, or ozone wash, and irradiation have been applied to minimally processed fresh produce (Ahvenainen 1996; deDaza and others 1996; Hoover 1997, Xu 1998).

Irradiation has gained attention as an effective tool for assuring food safety (Thayer and Rajkowski 1999). Irradiation delays ripening, inhibits growth and sprouting, and disinfects fresh produce (Kader 1986). Application of irradiation to minimally processed carrots and lettuce can extend shelf-life (Chervin and Boisseau 1994; Hagenmaier and Baker 1997, 1998). However, textual changes induced by irradiation is still one of the main limiting factors for its use in fresh produce. Tissue softening with increasing doses of irradiation over critical thresholds (Massey and Bourke 1967). Radiation-induced softening has been attributed to breakdown of cell wall constituents such as pectin, cellulose and hemicellulose, and alteration of semi-permeable membranes, which result in structural weakening and loss of turgor, respectively, in tissues (Kertesz and others 1964). The extent of the softening depends on dose levels, cultivar and storage period. Beneficial effects of irradiation on shelf-life and quality including texture of whole apples after long-term storage have been reported. For instance, Chuanyao and others (1993) irradiated ‘Golden Delicious’ apples and found that firmness of apples irradiated with 0.3 to 0.9 kGy was higher than that of non-irradiated apples with increasing storage time (63 to 76 d). Despite the initial softening caused by irradiation, irradiated apples softened at a much slower rate than did non-irradiated fruit during storage (Massey and others 1964), and Olsen and others (1989) found that irradiation doses of 1 kGy resulted in acceptable apple quality for marketing after 11-month storage.

Calcium plays an important role in maintaining the cell wall structure in fruits through cross-linking the pectic acid in the cell wall to form calcium pectate (Ferguson 1984). Koves and others (1988) found that shelf life of apples and pears was increased by irradiation (1 kGy) combined with calcium.

Irradiation stimulated respiration, but inhibited ethylene production in apple slices from different cultivars (Gunes and others 2000). Investigation of the influence of irradiation on the texture of cut processed apples is needed for determining the feasibility of using this technology on minimally processed apples. Thus, the purpose of our research was to quantify the textural changes in fresh-cut apple slices resulting from irradiation, to determine the mechanism of these changes, and to investigate the role of irradiation parameters and calcium on the irradiation-induced softening of apple slices.

Materials and Methods

Reagents
Calcium chloride, citric acid, sodium tetraborate and indigo carmine (a dye for histological studies) were purchased from Fisher Scientific (Pittsburgh, Pa., U.S.A.). L-ascorbic acid, L-cysteine, galacturonic acid, oxalic acid, and m-hydroxydiphenyl were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Ammonium oxalate was obtained from General Chemical Co. (New York, N.Y., U.S.A.).

Plant material and slice preparation
‘Delicious’ apples (strain Red Chief) were harvested from the Cornell University Orchard in Ithaca, N.Y., U.S.A., cooled overnight and then stored under a controlled atmosphere (CA) conditions (2% O\(_2\), 2% CO\(_2\), balance N\(_2\); 95% RH and 0.5 °C) until use. Apple fruits were selected for uniform size, peeled, cored and each sliced to eight wedges into cold water with a hand-peeler and slicer. The slices were randomly placed in pouches prepared from a high-barrier copolymer film (CVP Systems, Inc., Carol Stream, Ill., U.S.A.) consisting of a sandwich of 0.8 mil nylon, 1.2 mil ethyl vinyl acetate, and 1.2 mil surlyn. The gas transmission rates (cc.m\(^{-2}\).24 h\(^{-1}\)) of the film provided by the manufacturer were 28 to 38 for O\(_2\), 4 to 7 for N\(_2\), and 108 to 128 for CO\(_2\). The pouches were then vacuumed and sealed with a Multivac packaging machine (Model AGW; Koch, Kansas City, Mo., U.S.A.).
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Irradiation

Apple slices were irradiated using a 10,000-Curie Co\(^{60}\) source at the Ward Laboratory of Nuclear Engineering at Cornell University. The irradiator was a point source (179 mm long and 6.7 cm in diameter) with twelve Co\(^{60}\) pencils. The dose levels and dose rates were controlled by the time of exposure and the distance from the source. Dose rate was calculated from an exposure rate versus radial distance from the source curve, which was generated by a standard ionization chamber dosimeter. Packages of slices were placed in upright position in a metal rack installed into styrofoam containers. Temperature of the slices during irradiation was maintained approximately at 3 °C with an ice-water bath at the bottom of the containers. Control samples were treated identically in each experiment but kept outside the irradiation chamber.

Firmness measurement

Firmness of apple slices by back-extrusion cell was measured according to Bourne and Moyer (1968). The cell, which consisted of a stainless steel cup of 5.8 cm i.d. × 9.7 cm internal height, and a plunger with a diameter of 4.9 cm, was installed in an Instron Universal Testing Machine (Model 1122; Instron Corp., Canton, Mass., U.S.A.). Each slice (wedge) was cut into two pieces and approximately 70-g tissue was placed in the cup, and extruded with the plunger at a speed of 50 mm.min\(^{-1}\). The downward movement of the plunger was reversed at 1.0 cm from the bottom of the cup. Firmness was taken as the maximum force recorded on the chart and expressed in newton (N). The measurement was duplicated for each treatment. The puncture test was carried out by a flat surface pressed in newton (N). The measurement was duplicated for each treatment, and irradiated at doses of 0.09, 0.15, 0.22, 0.30, 0.34, 0.58, 0.88, and 1.17 kGy. Firmness of slices was measured in duplicate by a back-extrusion cell after 2 h (day 0), 3 d and 6 d storage at 5 °C in air following irradiation. For determination of the influence of irradiation on cell wall pectin, the same experiment was repeated, and the treated slices were frozen in liquid N\(_2\) and stored at –20 °C until analyzed.

Effect of irradiation on firmness and cell wall pectin

Duplicate packages of apple slices were selected randomly for each treatment, and irradiated at doses of 0.09, 0.15, 0.22, 0.30, 0.34, 0.58, 0.88, and 1.17 kGy. Firmness of slices was measured in duplicate by a back-extrusion cell after 2 h (day 0), 3 d and 6 d storage at 5 °C in air following irradiation. For determination of the influence of irradiation on cell wall pectin, the same experiment was repeated, and the treated slices were frozen in liquid N\(_2\) and stored at –20 °C until analyzed.

Pectin analysis

Pectin fractions of the slices were extracted and measured after 2 h (day 0) and 6 d storage at 5 °C in air following the methods described by Yu and others (1996). Slices were thawed and homogenized with a coffee grinder (Cuisinart® model DCG-20; East Windsor, N.J., U.S.A.). The homogenized tissue (5 g) was mixed with 30 mL boiling ethanol in a 50-mL centrifuge tube, and stirred thoroughly with a glass rod in a boiling water bath for 10 min. After cooling in ice-water the homogenate was centrifuged at 12,100 × g for 10 min in a refrigerated ultracentrifuge (Model RC2-B; Sorvall Centrifuge, Norwalk, Conn., U.S.A.). The alcoholic supernatant was discarded. The precipitate was extracted again, and the residue was dried for 24 h in a conventional oven at 35 °C in an Al weighing dish, and designated as alcohol-insoluble solid (AIS). AIS (80 mg) was placed in a 50-mL centrifuge tube with 20 mL distilled water and stirred with a glass rod for 1 min, and centrifuged at 12,100 × g for 10 min. Water-soluble pectin (WSP) was obtained by filtering the extract through Whatman No. 1 filter paper. The extraction was repeated again and the supernatants combined and diluted to 100 mL in a volumetric flask.

For extraction of oxalate-soluble pectin (OSP) the residue from the WSP extraction was dispersed in 20 mL water containing 0.25% ammonium oxalate and 0.25% oxalic acid. The sample was refluxed in boiling water for 1 hr, and then centrifuged at 12,100 × g for 10 min. The extract was filtered through Whatman No. 1 filter paper. The extraction was repeated and the supernatants combined and diluted to 100 mL in a volumetric flask.

For measurement of total pectin (TP) 5 mg AIS was placed in a 30-mL beaker containing a magnetic stirrer, and 2 mL concentrated H\(_2\)SO\(_4\) was added. The mixture was stirred gently, and then stirred on a stir plate while 1.0 mL distilled water was added dropwise. Stirring was continued for 30 min until dissolution of AIS was complete. The dissolved sample was filtered through glass wool into a 25-mL flask, and diluted to volume with distilled water. The solution was stored at 4 °C until use, and filtered through glass wool before analysis.

Pectin was quantified using the m-hydroxydiphenyl method as described by Kintner and Van Buren (1982). Extract (1 mL) was placed in a test tube, and 6 mL H\(_2\)SO\(_4\)/tetraborate solution (0.0125 M sodium tetraborate in concentrated H\(_2\)SO\(_4\)) was added in an ice-water bath. The solution was vortexed. Duplicate samples were prepared for each sample with a corresponding blank. The tubes were heated in boiling water precisely for 5 min and immediately placed in ice water to cool. An aliquot of 0.15% m-hydroxydiphenyl (0.1 mL) was added to samples to develop color, and 0.1 mL NaOH (0.5%) was added to the blank tubes. All samples and blanks were vortexed and allowed to stand for 15 min at room temperature. Absorbance at 520 nm was measured using a spectrophotometer (Model DU 7400; Beckman, Columbia, Md., U.S.A.). A solution of 1 mL distilled water, 6 mL H\(_2\)SO\(_4\)/tetraborate, and 0.1 mL NaOH was used as the reagent blank to zero the instrument. A standard curve was prepared using galacturonic acid solutions to quantify the pectin content in samples.

Effect of irradiation atmosphere and dose rate

Packages of slices were vacuumed and then flushed with 100% N\(_2\), 100% O\(_2\), or air before being sealed, and irradiated in duplicate at doses of 1 and 3.12 kGy at two different dose rates: 0.4 and 2 kGy.h\(^{-1}\). Firmness of slices was measured by back-extrusion cell as described above after 2 h (day 0), and 3 d and 6 d storage at 5 °C in air.

Effect of calcium on irradiation-induced softening

Apple slices were dipped in 25 mM L-cysteine with or without 0.5% (w/v) CaCl\(_2\) for 5 min vacuum-packaged, and irradiated in duplicate at doses of 2.5 and 5 kGy. The slices were then weighed and placed in 500-mL glass jars, which were attached to a flow-through system of 1.7% O\(_2\) and 4.9% CO\(_2\) (balance N\(_2\)) gas mixture, obtained by mixing CO\(_2\), N\(_2\), and air in a flowboard. Gases were humidified and filtered through a 0.45-mm membrane filter before entering the jars. Duplicate jars were sampled weekly to measure firmness of slices for up to 4 w.

In a second experiment, slices were dipped in 0, 0.5, 1, and 2% CaCl\(_2\) in an antibrowning solution (1% ascorbic acid and 0.5% citric acid) for 5 min. Vacuum-packaged slices were...
Irradiated in duplicate with 1 kGy. Firmness of the slices was measured as above after 2 h (day 0) and 3 d storage in air at 5 °C following irradiation.

Infiltration of calcium was carried out with thin apple slices. Apples were sliced by a hand-operated slicer (The Eagle Tool & Mach. Co., Springfield, Ohio, U.S.A.) into rings of 3-4 mm thickness. The slices from each apple were distributed among the treatments randomly, and immerged in 0, 2, and 4% CaCl₂ solutions in the antibrowning solution as above. The slices were submerged in the solutions in a desiccator, vacuumed to a pressure of 50.8 kPa, and held at that pressure for 5 min. Otherwise the slices were dipped in the solutions under atmospheric pressure for the same duration. Treated slices, then, were irradiated in duplicate vacuum packages at 0 and 1 kGy doses, and their firmness was measured by puncture test as above. For each treatment 10 slices were used to determine the firmness.

### Statistical analysis

The General Linear Model procedure was used to determine effects of irradiation dose, calcium, dose rate, atmosphere, and postirradiation storage period and their interactions in a complete randomized design using Minitab, release 12 (Minitab Inc., State College, Pa., U.S.A.). Tukey’s and Fisher’s multiple comparisons at 95% level were performed for the comparison of the levels for each factor.

### Results and Discussion

**Irradiation reduced firmness in a dose-dependent manner** (Figure 1). As dose increased firmness decreased. Multiple comparison of the individual dose levels revealed that the loss of firmness was significant at doses above 0.34 kGy; change in firmness caused by doses at or below 0.34 kGy was not significant (p > 0.05). Boyle and others (1957) reported threshold doses ranged from $4.2 \times 10^3$ to $10^7 \times 10^3$ r (0.039 to 0.954 kGy) for softening of whole apples depending on cultivar. ‘Delicious’ apples were not investigated, but the average threshold dose over several cultivars was $34.3 \times 10^3$ r (0.32 kGy), which is very close to 0.34 kGy found in our experiments. Firmness of slices were slightly higher after 3 d and 6 d cold storage (Figure 1).

Softening of slices induced by irradiation was associated with increased water-soluble pectin and decreased oxalate-soluble pectin content (Figure 2). Total pectin content was unaffected by irradiation. Both the water-soluble and the oxalate-soluble pectin fractions were significantly correlated with the decrease in firmness by irradiation ($r = -0.974$ and -0.954, respectively). Solubilization of pectin by irradiation has been reported for whole apples and strawberries (Kertesz and others 1964; Yu and others, 1996). Kertesz and others (1964) reported degradation of pectin in solution at doses of 8300 r (0.077 kGy), and threshold doses for the degradation of pectin and softening of apple tissues were similar. Yu and others (1996) found a significant correlation between the firmness and oxalate-soluble pectin in irradiated strawberries, but no correlation between water-soluble pectin and firmness.

Dose rate affected the textural response of slices on day 0 (Table 1): higher dose rate resulted in smaller loss of firmness than lower dose rate (p = 0.009). However, the effect of dose rate was not significant after 3-d and 6-d storage of slices in air at 5 °C. Dose rates that are lower than 0.4 kGy.h⁻¹ and higher than 2 kGy.h⁻¹ may have a significant effect on the firmness of irradiated slices during storage. However, these dose rates may not be practical since higher dose rate causes higher variation in absorbed dose level within slices, and lower dose rate requires excessive treatment periods. Irradiation at 1 and 3.17 kGy resulted in significant softening compared to nonirradiated slices (Table 1). Oxygen concentration during irradiation and days in storage did not affect firmness of irradiated slices (Table 1). A lack of storage time and irradiation atmosphere effects suggests that softening of irradiated apple slices is probably a direct physical effect rather than one mediated by free radicals. Conditions during irradiation treatment such as dose rate, temperature, and O₂ level in the surrounding atmosphere can influence responses of biological systems to irradiation (Taub 1983). As opposed to our observation with apple slices, protective effects of anoxia against textural changes in irradiated nectarines, peaches, and pears have been reported (Somogyi and Romani 1964). Since the dose rate and the irradiation atmosphere did not have an effect on firmness of slices it may be possible to modify these parameters in order to optimize microbiological and physiological responses. Different irradiation temperatures were not tested because slices would be negatively affected above refrigeration temperature.

Irradiation at 2.5 and 5 kGy resulted in softening of slices stored under CA for 4 w irrespective of calcium treatment.

### Table 1—Firmness (N) of apple slices irradiated at 0, 1, and 3.17 kGy as affected by 0.4 and 2 kGy.h⁻¹ dose rate, O₂ concentration in irradiation atmosphere (balance N₂), and post-irradiation storage day at 5 °C in air.

<table>
<thead>
<tr>
<th>Time (d)</th>
<th>Dose and dose rates</th>
<th>0 kGy</th>
<th>1 kGy</th>
<th>3.17 kGy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.4 kGy.h⁻¹</td>
<td>2 kGy.h⁻¹</td>
<td>0.4 kGy.h⁻¹</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>1552±a</td>
<td>913±c</td>
<td>1042±c</td>
</tr>
<tr>
<td>21</td>
<td></td>
<td>1549±a</td>
<td>1015±b</td>
<td>1086±b</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>1621±a</td>
<td>1076±b</td>
<td>1081±b</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0</td>
<td>1611±a</td>
<td>1049±b</td>
</tr>
<tr>
<td>21</td>
<td></td>
<td>1652±a</td>
<td>1052±b</td>
<td>1052±b</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>1613±a</td>
<td>1010±b</td>
<td>1039±b</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>0</td>
<td>1675±a</td>
<td>1128±b</td>
</tr>
<tr>
<td>21</td>
<td></td>
<td>1677±a</td>
<td>1020±b</td>
<td>1066±b</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>1675±a</td>
<td>1113±a</td>
<td>1012±b</td>
</tr>
</tbody>
</table>

Numbers with different letters within each time row are significantly different at 95% confidence level.

*Figure 1—Firmness (N) of apple slices irradiated at doses of 0 to 1.2 kGy and stored for 0, 3, and 6 d post-irradiation at 5 °C in air.*
Gamma Irradiation of Apple Slices...

Gamma irradiation of apple slices resulted in limited but significant improvement on firmness during 4-w storage (p = 0.000). Postirradiation storage period under CA did not affect firmness. The firmness of slices treated with both calcium and irradiation was lower than nonirradiated control slices (p < 0.05). Dipping apple slices in up to 2% CaCl₂ combined with an antibrowning mixture (ascorbic and citric acids) resulted in firmer slices (p = 0.002), but this improvement was small compared to the loss of firmness by irradiation at 1 kGy (Figure 4). Addition of calcium to an antibrowning solution has been reported to increase firmness of fresh apple slices (Ponting and others 1971; Lee and Smith 1995). Divalent calcium ions cross-link with pectic acid and thus maintain the integrity of the middle lamella, which results in retention of firmness (Ferguson 1984). Kovacs and others (1988) showed that calcium had a role in stabilizing cell membranes to a greater extent than its role in middle lamella region in irradiated whole apples.

The inability of calcium chloride to prevent irradiation-induced softening may be due to limited penetration into the tissue. We measured the penetration into the tissue by adding indigo carmine to the CaCl₂ solution and measuring dye penetration. Dipping wedges in solution for 5 min resulted in limited (less than 0.5 mm) penetration from the surfaces. Vacuum infusion at 50.8 kPa for 5 min resulted in approximately 2 mm penetration of calcium solution into apple wedges. However, this penetration was also limited compared with the total thickness of the wedges, 1 to 2 cm, and thus it had limited effect on firmness. The firmness of the wedges were measured by the back extrusion method that involve total smashing of the tissues. The firmness recorded in this procedure depends on the all parts of the wedges most of which had no CaCl₂. Therefore, thin apple slices (3 to 4 mm thick), higher CaCl₂ levels (0 to 4%), and vacuum-infusion were evaluated.

Both dipping and vacuum-infusion of CaCl₂ solution reduced irradiation-induced softening in thin slices (Table 2).
Increased CaCl₂ concentration increased firmness. The firmness of the calcium-treated irradiated slices was not different from samples evaluated immediately after slicing (Table 2). However, firmness of calcium-treated irradiated samples was somewhat less than the corresponding nonirradiated controls with the same calcium treatment. Comparison of vacuum-infused slices with dipped slices showed the former to be less firm (p = 0.001). This may be explained by the fact that infiltration results in entry of solution in intercellular spaces causing solubilization of some pectin as well as alteration of osmotic balance cell membranes (Stow 1989; Glenn and Poovala 1990). Thus, an effective procedure for calcium penetration into tissue is needed for thicker slices, like wedges, to prevent or minimize irradiation-induced softening. Simple vacuum-infusion was ineffective due to its undesirable side effect of waterlogging and thus softening. Further investigation with pressure infiltration may be useful since this method results in no entrapment of solution in tissues after the treatment. Irradiation with electron beams may have less softening effect due to its lower penetration than gamma irradiation from Co⁶⁰ (Niy 1989; Radomyski and others 1994). Softening associated with the electron beam irradiation may be confined to surface of the slices and eliminated by a normal calcium treatment. Therefore, electron beam irradiation can effectively inactivate foodborne pathogens on the surface of the slices, which is one of the main purpose of the treatment, with minimum effect on the firmness of slices.

**Conclusion**

Irradiation at doses above 0.34 kGy reduced firmness of minimally processed apples. Irradiation-induced softening was associated with higher water-soluble and lower oxalate soluble pectin contents. Calcium-dipping had limited effect on firmness of irradiated apple wedges, but prevented irradiation-induced softening of thinner apple slices. Therefore, softening remains as one of the main constraint for application of gamma irradiation to minimally processed apples until an appropriate calcium infiltration procedure is developed, which enables complete penetration of calcium to inner tissues without any undesirable side effects. In addition, the effect of CaCl₂ treatment and irradiation on flavor of slices needs to be determined.

**References**


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**Authors Gunes and Hotchkiss are with the Dept. of Food Science and author Watkins is with the Dept. of Horticulture, Cornell Univ. Direct inquiries to author Hotchkiss, Dept. of Food Science, Stocking Hall, Cornell Univ., Ithaca, NY 14853 (E-mail: jhh3@cornell.edu).**