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Causes of irradiation-induced softening in peaches

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ABSTRACT

Acceleration of ripening has been offered as a cause of irradiation-induced loss of firmness in peaches (*Prunus persica*). This work aimed to correlate softening with biochemical and physiological responses induced by irradiation in fresh peaches during subsequent storage. Freshly harvested and cooled 'Mid Pride'peaches were treated with gamma irradiation at 1030 Gy, then stored at 1 °C for 7 days, and at 22 °C for another 6 days, during which time ripening and biochemical parameters were measured. The changes in respiration rate, ethylene, firmness, total soluble solids (TSS), titratable acidity (TA), water-soluble pectin (WSP) and activity of polygalacturonase (PG) and pectin methylesterase (PME) due to treatment and storage were evaluated. Firmness was significantly reduced immediately following irradiation treatment, whereas total pectin remained unaltered. WSP, however, increased upon irradiation and also during ripening. Despite an increase in respiration rate, ethylene evolution, TSS and TA were unaffected by irradiation. PG activity was not affected by irradiation, but, both PME and electrolyte leakage increased following irradiation treatment. For early ripe irradiated 'Mid Pride' peaches, softening during storage appears to be related to pectin hydrolysis, PME activity and electrolyte leakage, and not associated with ethylene evolution or exo-PG activity.

1. Introduction

Peaches destined from the USA to Mexico are treated with methyl bromide fumigation for disinfestation of insect pests, but methyl bromide use is slated for elimination under the Montreal Protocol (US EPA, 2017). Irradiation can be used on peaches which can tolerate the low doses required for phytosanitary treatment (McDonald et al., 2013). In 2015 and 2016, peaches were the first fruit to be exported from the USA to Mexico under a irradiation workplan.

The major impact of irradiation on peaches is loss of firmness, with tolerance depending on dose, cultivar and ripening stage upon treatment (Ahmed et al., 1966). Sensory perception of loss of firmness is manifested at doses of 600 Gy and higher (McDonald et al., 2012). Prior studies have associated this immediate change in texture to a breakdown of pectin structure (Somogyi and Romani, 1964; Braddock et al., 1966). Hussain et al. (2008), however, observed that firmness continued to decrease in irradiated peaches during storage especially under ambient temperatures. Peaches are climacteric fruits that undergo ripening and concomitant softening soon after harvest, thus, it has also been suggested that irradiation accelerates the ripening process and that the activation of ethylene-mediated hydrolytic enzymes, such as pectin methylesterase (PME) and polygalacturonase (PG) can contribute to loss of firmness in irradiated peaches during storage (McDonald et al., 2012). In climacteric fruit, the impact of irradiation on firmness is complex and highly variable depending on fruit and maturity stage. For example, D'Innocenzo and Lajolo (2001) found that irradiation inhibited polygalactouronase activity in papaya, slowed the process of ripening and helped maintain firmness. Silva et al. (2012) found that mangoes irradiated at 500 Gy had higher levels of pectin in the middle lamella as compared to non-irradiated mangoes and mangoes treated at 1000 Gy. Ahmed et al. (1972) and Hussain et al. (2008) found that while irradiation resulted in an immediate loss of firmness, ripening of the fruit had a greater impact on texture, suggesting that ripening related enzymatic activity may also be influenced by irradiation.

In peaches, ethylene mediated ripening processes have not been evaluated during storage after irradiation treatment. Thus, the objective of this study was to correlate the loss of firmness in irradiated peaches to physiological responses such as changes in ethylene, respiration rate, activity of hydrolytic enzymes as well as cell membrane damage during storage and ripening. Ripening was determined by measurement of ethylene production and respiration rate, titratable acidity and soluble solids content, while textural changes were monitored using a texture analyzer and correlated to changes in pectin and activity hydrolytic

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enzymes. A dose of 1000 Gy was selected to exaggerate the effects so that changes can be measured. Peaches were kept under refrigeration for one week after treatment to simulate shipment to Mexico from the US, then brought out to ambient conditions for ripening and to mimic retail conditions.

2. Material and methods

2.1. Fruit source, irradiation treatment, and storage conditions

Peaches (*Prunus persica* L.), cv. Mid Pride, were harvested on July 16, 2017, from an orchard at the Great Park, Irvine, California. The fruit were selected according to uniform size and skin background color changing from green to yellow, considered as the onset of ripening for this cultivar. The fruit were transported to Chapman University (12 km), dipped for 10 min in a solution of 100 mg L^{-1} of active chlorine at 3 °C, prepared from dilution of sodium hypochlorite 5.25% in distilled water and pH adjusted to 7.0 with HCl, then allowed to dry for 1 h. Fruits were randomly sorted into two groups and placed in a cold chamber at 1 °C for 72 h prior to irradiation treatment.

Fruits were irradiated using Cesium 137 as gamma irradiation source at the Nuclear Science Laboratory, Department of Chemistry at University of California, Irvine. Fruits were placed in a custom built three shelf sample holder at a distance 100 mm from the irradiation source to receive a dose rate of ~ 1 kGy h^{-1}. Midway through the process, the fruit was rotated 180° to ensure uniformity. Gammachromic dosimeter film (Gafchromic HDV2, Ashland Specialty Ingredients, Bridgewater, NJ, USA) calibrated against alanine dosimeters (Farwest Technology, Inc., Goleta, CA, USA) were used to construct a dose map. During treatment, dosimeters were placed at minimum and maximum dose locations which had been previously determined by dose mapping. The achieved doses ranged from 820 to 1175 Gy to give a Dmax/Dmin ratio of 1.4, and an average absorbed dose of 1030 Gy. The irradiation chamber was not temperature controlled, so an increase to 5–7 °C was measured in the fruit following treatment.

After irradiation, fruits were transported back to Chapman University, in styrofoam box filled with ice packs, and placed in cold chamber at 1 °C 95% RH. For evaluation on day 0, which occurred 2 h after irradiation, one third of the fruits was allowed to warm to a temperature at 22 °C prior to analysis of ethylene evolution and respiration rates, texture, electrolyte leakage, and physical-chemical parameters. Fruits were peeled, chopped, immediately frozen with liquid nitrogen and kept in −80 °C for enzyme, sugar and MDA analyses. Remaining fruits were maintained at 1 °C for 7 days, then at 23 ± 2 °C for another 6 days to simulate conditions during distribution then retail and consumer use. The same sampling procedures were carried out on days 7 and 13.

2.2. Respiration and ethylene production rates

Carbon dioxide and ethylene production were measured daily during ripening using a static system as described by Sea et al. (2015). Three peaches from each treatment were weighed and placed in individual 1.89 L glass jars, 4 jars per treatment. The jars were sealed with size 15 rubber stoppers (Plasticoid Company, Elkton, MD, USA), equipped with 2 holes through which two Polyflo plastic tubes (California Equipment, Sacramento, CA, USA) were placed. Latex tubing (Primeline Industries, Akron, OH, USA) connected the 2 plastic tubes that allowed for secure gas extraction using a syringe. Each jar was allowed to equilibrate for 1 h, and 1 mL of gas was extracted at intervals of 60, 65, and 70 min. The equipment used was a SRI 8610C gas chromatograph (GC) (SRI Instruments, Torrance, CA, USA) equipped with a thermal conductivity detector for measurement of carbon dioxide and a flame ionization detector (FID) to detect C_2H_4, both maintained at 150 °C. Injections were direct on a stainless steel 6' Hayesep-D packed column heated to 80 °C. Hydrogen was used as the carrier and fuel gas at a flow rate of 15 mL min^{-1}. Oxygen for the FID was provided by a built-in air pump, operating at 250 mL min^{-1}.

The gases were quantified by peak area in relation to the area of the standards used for the calibration curves and expressed as mL CO_2 kg^{-1} h^{-1} and μL C_2H_4 kg^{-1} h^{-1}. Standards for carbon dioxide (CO_2) and ethylene (C_2H_4) were prepared in airtight 15.24 cm x 15.24 cm gas sampling bags equipped with an Alltech general-purpose septa (Fisher Scientific, Hampton, NH, USA). Air was removed from the gas bags by vacuum. Each bag was then filled with inert nitrogen gas (N_2) (Air Liquide America Specialty Gases, LLC, Plumsteadville, PA, USA) and vacuumed again. Starting with a custom gas mixture of 20,000 ppm CO_2 and 101 ppm C_2H_4 (Air Liquide America Specialty Gases, LLC, Plumsteadville, PA, USA), dilutions ranging from 101 to 20,000 ppm for CO_2 and 0.02–101 ppm for C_2H_4 were prepared for a standard curve using nitrogen gas as the diluent. A 10 mL BD Luer-Lok Tip syringe equipped with a 1-way male slip stop cock, and a needle attachment (Cole Parmer, Vernon Hills, IL, USA) was used to extract the gases from the gas bags.

2.3. Titratable acidity (TA) and Total soluble solids (TSS)

Fruit pulp was blended for 30–45 s in four separate replicates of 3 fruits per treatment (Kitchen Aid; St. Joseph, MI, USA), and filtered through two layers of cheesecloth. TA was measured using 5 mL of filtered juice in an automatic titrator (HI-84532, Hanna Instruments, Woonsocket, RI, USA), and expressed as g.100 mL^{-1} of malic acid. TSS was measured in the filtered juice using a digital refractometer (Pocket Pal α ATAGO, Tokyo, Japan) and expressed as °Brix.

2.4. Texture

Puncture tests were performed at two freshly peeled sections on opposite sides in the equatorial zone of each fruit using a texture analyzer (Model TA.XT Plus, Stable Micro Systems, Inc., Surrey, UK) equipped with Exponent 6.1.10.1 software. A 2 mm cylinder probe was used, with a penetration depth of 10 mm, and test speed of 2 mm s^{-1}. Firmness was measured as the maximum force (N) and the area of work below the force x distance curve was also recorded. The linear distance, representing crispiness, was also recorded between 1 and 5 s of the test. Puncture tests were performed on four replicates of three fruits each.

2.5. Pectins

Extraction: Total and water-soluble pectins were extracted according to McCready and McComb (1952). For extraction of both water-soluble and total pectins, 5 g of mesocarp was homogenized in a tissue homogenizer (Pro-250, Pro Scientific, Oxford, CT, USA) with 25 mL of 95% ethanol. Samples were shaken (I2400 Incubator Shaker, New Brunswick Scientific, Co., Inc, Edison, NJ, USA) for 1 h and allowed to stand for 12 h. The pulp was washed three times with 30 mL of 95% ethanol and the residue discarded. For total pectin, the residue was re-suspended in 70 mL of 0.5% tetrassodium EDTA to sequester cations, the pH raised to 11.5, and allowed to stand for 30 min for pectin de-esterification. The mixture was acidified to pH 5.5 with glacial acetic acid for depolymerization with 0.1 g of pectinase (Pectinase from *Aspergillus niger*, Sigma Aldrich, Co., St. Louis, MO, USA), and shaken for 1 h, prior to adjustment of the volume to 100 mL. For water-soluble pectin, the ethanol washed residue was re-suspended in 40 mL of deionized water, shaken for 2 h and filtered. For determination of total and water-soluble pectin, a modified method of Bitter and Muir (1962) was followed. A 100 μL of filtered extract was diluted with 900 μL of water. Samples were cooled to 3 °C and titrated in ice with 3 mL of concentrated sulfuric acid (Sigma-Aldrich, Co. St. Louis, MO, USA), containing 0.96% sodium tetraborate. Samples were heated in boiling water for 10 min and cooled in ice bath to about 20 °C for addition of 100 μL of 0.15% carbazole solution. After thoroughly mixing, samples were heated again