Fall 12-2021

Gamma and X-ray Irradiation Improve Postharvest Shelf-Life in 'Bartlett' Pears

Matthew Garcia
Chapman University, mattgarcia@chapman.edu

Gamma and X-ray Irradiation Improve Postharvest Shelf-life in 'Bartlett' Pears

A Thesis by

Matthew Troy Garcia

Chapman University

Orange, CA

Schmid College of Science and Technology

Submitted in partial fulfillment of the requirements for the degree of

Master of Science in Food Science

December 2021

Committee in charge:

Anuradha Prakash, Ph.D., Chair

Lilian Were, Ph.D.

Hagop Atamian, Ph.D.

The thesis of Matthew Troy Garcia is approved.

Anuradha Prakash, Ph.D., Chair
Professor, Director, Food Science Program
Chapman University | Schmid College of Science and Technology

Lilian Were

Digitally signed by Lilian Were
Date: 2021.12.06 08:24:43 -08'00'

Lilian Were, Ph.D.
Professor, Food Science Program
Chapman University | Schmid College of Science and Technology

Hagop Atamian, Ph.D.
Assistant Professor, Biological Sciences
Chapman University | Schmid College of Science and Technology

November 2021

Gamma and X-ray Irradiation Improve Postharvest Shelf-Life in 'Bartlett' Pears

by Matthew Troy Garcia

ACKNOWLEDGEMENTS

A special thank you to Dr. Anuradha Prakash and Dr. Lilian Were for their excellent guidance, instruction, and inspiration through the research and writing process. I also thank my friends, colleagues, and peers who helped and supported this effort at all of its many stages. I dedicate this thesis to my grandmother, Charlotte Garcia, mother, Martha Warren, and father, Secundino Garcia. Their motivation and love have been a shining light in the periods when I truly needed it the most.

ABSTRACT

Gamma and X-ray Irradiation Improve Postharvest Shelf-Life in 'Bartlett' Pears

by Matthew Troy Garcia

The objective of this study was to investigate the effects of gamma and x-ray treatment on 'Bartlett' pear physiology during post-harvest storage. Freshly harvested 'Bartlett' pears were irradiated with gamma irradiation at 0.412 and 0.819 kGy and x-ray irradiation at 1.03 and 1.94 kGy, then stored at 0-1 ºC and 95% relative humidity. The pears were removed from cold storage at 45 and 75 days and allowed to ripen at room temperature for four and two days, respectively. Respiration rate and ethylene production were measured during fruit ripening. Color, firmness, titratable acidity, soluble solids contents, and occurrence of superficial scald was measured at the climacteric peak. Ripening was accelerated in all pears during storage, and by day 75, the fruit ripened in three days compared to seven days before storage. Ethylene production was decreased, and respiration rate was increased in the irradiated fruit in a dose-dependent manner. Superficial scald developed in pears only after 75 days of cold storage. Superficial scald was lower in pears treated at the higher doses by x-ray as compared to the lower doses and the control pears, although concentrations of alpha-farnesene and conjugated trienes, considered precursors to superficial scald, were not affected This research suggests that the formation of superficial scald can be influenced by factors other than alpha-farnesene and conjugated trienes. Irradiation also suppressed fungal decay in x-ray irradiated pears. Despite being treated at a higher dose, the x-ray treated fruit maintained a firmer texture and delayed yellowing compared to gamma treated fruit. This research shows that the higher doses of irradiation were more effective at lowering ethylene production and decreasing superficial scald development with little impact on the quality of 'Bartlett' pears.

Keywords: α-farnesene, ethylene, gamma, postharvest, superficial scald, x-ray

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

Abbreviation	Meaning
1-MCP	1-Methylcyclopropane (PubChem CID: 11657)
ACC	1-methylcyclopropane-1-carboxylic acid
ACO	1-aminocyclopropane-1-carboxylate oxidase
ACS	1-aminocyclopropane-1-carboxylate synthase
AF	Alpha-farnesene
CA	Controlled atmosphere
CT	Conjugated trienes
DPA	Diphenylamine (Pubchem CID: 11487)
DUR	Dose uniformity ratio
FID	Flame ionizing detector
PAL	Phenylalanine ammonia lyase
POD	Peroxidase
PPO	Polyphenol oxidase
SAM	S-adenosyl-L-methionine (Pubchem CID: 34755)
SSC	Soluble solids content
TA	Titratable acidity
TCD	Thermal conductivity detector

1 Introduction

'Bartlett' pears are one of the most popular pear varieties produced in the United States. In California, 'Bartlett' pears comprise 75% of the pear acreage and 90% of its tonnage (CFAITC, 2019). During postharvest storage, 'Bartlett' pears can develop physiological disorders such as internal browning and watery breakdown, as well as senescent scald, core breakdown, and superficial scald (Crisosto, 2020). These postharvest disorders in pears can lead to consumer dissatisfaction and economic loss (Pedreschi et al., 2007; Crisosto, 2020). Senescent scald appears as a brown to black discoloration on abnormally ripened fruit. This disorder develops as pears reach the end of their postharvest life and their tissues die. It begins as darkened spots around the calyx in a uniform pattern then spreads upward in the fruit (Whitaker, Villalobos-Acuña, Mitcham, and Mattheis, 2009; UC Davis, 2020).

Core breakdown occurs when 'Bartlett' pears are not cooled properly after harvest, are placed in higher temperatures than the recommended -1 to 0 ºC or are placed in storage longer than 2-3 months in air (UC Davis, 1971; Mitcham, Crisosto, and Kader, 1996; Crisosto, and Mitcham, 2020). This disorder develops as a softening of the core, and surrounding tissues with a brown appearance as the fruit starts to senesce, making it unfit to consume (UC Davis, 1971).

Superficial scald appears as localized brown or black patches on the skin of the fruit. This disorder occurs when pears are removed from extended storage under low temperature and warmed for several days (Lurie and Watkins, 2012). Superficial scald is associated with cell damage and death resulting from the accumulation of α-farnesene and its autoxidation products, conjugated trienols (CTols), in the peel of the fruit. The accumulation is mediated by slow ethylene production

during cold storage, which increases when the pears are stored at ambient temperature (Whitaker, Villalobos-Acuña, Mitcham, & Mattheis, 2009). α-farnesene synthase is a key enzyme for the biosynthesis of α-farnesene. Ethylene mediates α-farnesene production by inducing the gene expression of *AFS1* that codes for α-farnesene synthase (Pechous and Whitaker, 2004; Lurie and Watkins, 2012). Therefore, inhibition of ethylene can reduce α-farnesene synthase biosynthesis and indirectly limits the formation of superficial scald.

Ethylene is an essential hormone that mediates fruit ripening, particularly in climacteric fruit such as pears. Quality changes are also mediated by ethylene as pears ripen to exhibit a robust and pleasant aroma; a sweet taste; and a juicy, buttery texture (Mitcham, Crisosto, and Kader, 1996). For all three of these physiological disorders, decreasing ethylene production appears to reduce their incidence. 1-MCP, an ethylene inhibitor, is used extensively for apples and pears in the Pacific Northwest to delay ripening and reduce defects and extend shelf life. It can bind irreversibly to ethylene receptor sites, thereby inhibiting ethylene synthesis (Zhao et al., 2018). However, the use of 1-MCP as a postharvest treatment was discontinued in California in 2020 among some pear growers because of the unpredictable nature of the ripening process as a side effect of treated pears after they are pulled from cold storage (Fruit Growers News, 2020).

Although not used in California due to high cost, a controlled atmosphere comprising low oxygen and high carbon dioxide can extend the shelf life of pears by reducing superficial scald through control of ethylene production (Pedreschi et al., 2008; Zhi, Dong, and Wang, 2019; McClain, 2019). A controlled atmosphere reduces ethylene production by having low oxygen partial pressure (1-2.5 kPa) and high carbon dioxide partial pressure (0.5-5 kPa) (Lum et al., 2017). Because oxygen is required for ethylene biosynthesis, ethylene production can be reduced with low oxygen (Pedreschi et al., 2008).

Low dose irradiation used commercially as a phytosanitary treatment of fresh fruits can decrease ethylene production (Abolhassani, Caporaso, Rakovski, and Prakash, 2013; Sea, Rakovski, and Prakash, 2015; Melo et al., 2021). Irradiation-induced inhibition of ethylene production can reduce superficial scald in a variety of apples such as 'McIntosh' (Massey, Parsons, and Smock, 1964), 'Golden Delicious' (Al-Bachir, 1999), 'Gala' (Fan and Mattheis, 2001), and 'Granny Smith' (Melo et al., 2021). Irradiation-induced ethylene inhibition also inhibits the expression of α-farnesene synthase, the final enzyme in the α-farnesene pathway. Irradiation also inhibits ACC oxidase activity and reduces ethylene production, which is correlated with reducing superficial scald in 'Granny Smith' apples (Melo et al., 2021). However, irradiation can induce internal browning in pineapples (Jenjob et al., 2017) and 'Gala' (Fan and Mattheis, 2001) and 'Granny Smith' apples (Melo et al., 2021).

There are three modalities available for food irradiation – gamma, electron beam, and x-ray. Gamma rays are produced from radioactive sources such as Cobalt-60. Machine sources produce electron beams and x-rays through electron accelerators (CAC, 2003; FDA, 2019). Electron beams have a maximum penetration at 8 cm, while Gamma and x-ray have a high penetration depth of up to several feet (WHO, 1988). Gamma rays have a much lower dose rate than e-beam or x-ray, necessitating a longer treatment time to achieve the same dose. For example, a gamma source at 1.59 MeV has a dose rate of ~0.06-0.12 Gy/s. Electron beam at 10MeV has a dose rate of ~3,000 Gy/s, and x-rays at 5 MeV have a dose rate of ~100 Gy/s (Pillai, 2016). The dose rate of e-beam is the highest because e-beams can be highly focused, whereas gamma rays radiate in all directions (Fellows, 2017).

The impact of irradiation on fruit quality is highly dependent on the absorbed dose. Differences in dose rate, though, may also result in differences in physical and chemical

parameters. Irradiation reacts with food components, particularly water, and produces free radicals and ion pairs. The resulting reactions of the free radicals with each other and other molecules lead to changes in the structure and function of various molecules and reactions responsible for cell metabolism (McHugh, 2019). Dose rate can affect the production and neutralization of free radicals. Because the dose rate for x-ray is much higher than gamma, radiolytic products formed in such a short time have a higher probability of reacting with each other than with molecules of the irradiated system within the upper range of the dose rate of 10^9 Gy/s (Diehl, 1995; Diehl, 1999). Thus, free radicals may persist for a longer time in gamma-treated products as observed in (i) chrysanthemum plants that were gamma-irradiated with a total dose of up to 60 Gy at a dose rate of 0.5-2 Gy/h (Yamaguchi et al., 2008) (ii) mushrooms gamma-irradiated with 2 kGy at dose rates of 4.5 and 32 kGy/h (Beaulieu et al., 1999), and (iii) sweet potatoes gamma-irradiated with 1 kGy at a dose rate of 1-15.30 kGy/h (Lu et al., 1989).

Newer irradiation plants are likely to use electron beam and x-ray versus gamma because of the challenges (list them) associated with a radioactive source. However, few comparative studies evaluate the differences in fruit quality due to irradiation modality. Thus, the objectives of this study were:

1. Determine the effect of irradiation on ripening of ‘Bartlett’ pears after cold storage for three months in the air. Irradiation has been shown to delay ripening by decreasing ethylene production in treated pears after cold storage for two weeks, thus we hypothesize that irradiation of ‘Bartlett’ pears will decrease ethylene production, thereby delaying ripening in ‘Bartlett’ pears stored cold for three months.
2. Evaluate the incidence of physiological defects in irradiated ‘Bartlett’ pears. Ethylene production has been associated with an increase in α-farnesene accumulation and

subsequent development of superficial scald. A dose-dependent, irradiation-induced inhibition of ethylene will mitigate the development of superficial scald. Likewise, we expect to observe a decrease in the incidence of senescent scald and core breakdown as related to an irradiation-induced decrease of ethylene production.

3. Compare the differences in ripening and physiological defects between pears treated with gamma and x-rays. The higher dose rate of x-rays will allow radiolytic products, such as reactive oxygen species, to interact with each other and get neutralized, whereas, in gamma radiation, the lower dose rate will produce reactive oxygen species at a lower rate giving them a greater chance to interact with the food medium leading to a higher incidence of superficial scald.

2 Literature review

2.1 California ‘Bartlett’ pear tonnage and distribution

‘Bartlett’ pears (*Pyrus communis* L.) are the most common variety of summer pears grown in California (USAP, 2020). ‘Bartlett’ pears comprised about 26% or 4.4 million standard boxes of the 2019-20 pear harvest (FGN, 2020). In California, ‘Bartlett’ pears make up 75% of the pear acreage and 90% of its tonnage (CFAITC, 2019). The California Pear Advisory Board reported that California growers distributed 88% of harvested ‘Bartlett’ pears domestically in 2019 while Canada and Mexico received exports of 2% and 9% (**Figure 1**; McClain, 2019).

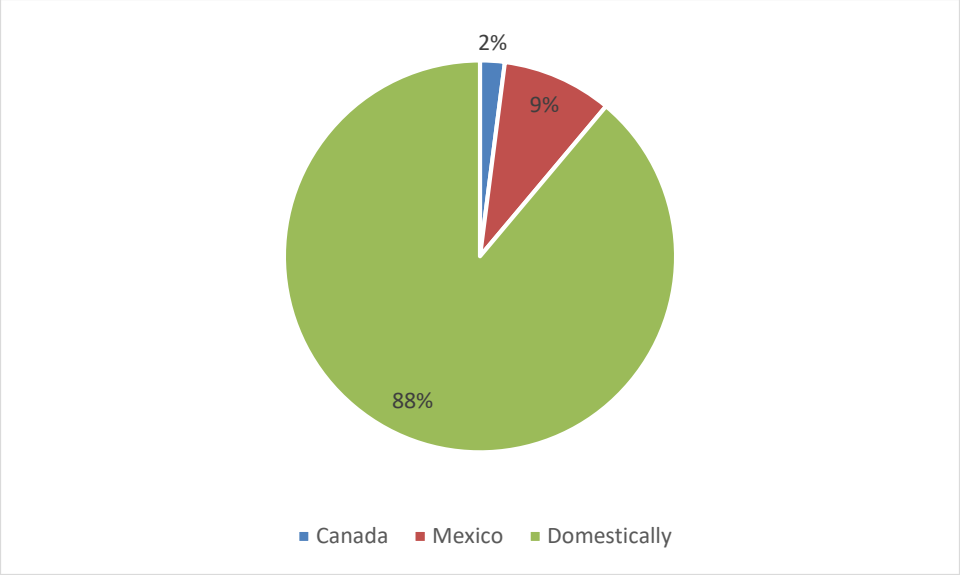

Figure 1. Exports of California ‘Bartlett’ pears to Canada, Mexico, and domestically (McClain, 2019).

2.2 Harvesting and postharvest handling of 'Bartlett' pears

Pears are climacteric fruits that do not fully ripen while on the tree. 'Bartlett' pears need to be harvested when at full maturity but before they are ripe. The most reliable indicator of maturity in pears is firmness. Maturity of California 'Bartlett' pears is determined using an index based on flesh firmness and soluble solids content (Mitcham, 1996). For example, mature 'Bartlett' pears with a fruit diameter between 2 3/8" - >2 ½" and firmness of 20.5 lb_f should have a minimum soluble solids content (SSC) of 11% (**Table 1**). The index can be further modified using skin color. If the skin color is yellowish-green, then there would be no firmness values required, and the SSC would not have any limits (Crassweller, 2017; Mitcham, 1996).

Table 1. Standards used to determine pear maturity (Crassweller, 2017; Mitcham, 1996).

Minimum Soluble Solids Content	Maximum Flesh Firmness (Pounds-Force)	
	Fruit Diameter	Fruit Diameter
	2 3/8" - 2 1/2"	>2 1/2"
<10%	19.0	20.0
10%	20.0	21.0
11%	20.5	21.5
12%	21.0	22.0
13%	[No Maximum]	[No Maximum]

Quality indices include appropriate shape, size, absence of insects, decay and defects, and good eating qualities characterized by sweetness, aroma, flavor, and texture (Mitcham, 1996).

Maturity at harvest is vital in achieving normal ripening during storage (Saquet, 2019). Pears that receive conditioning depend on when they were harvested. Conditioning is the use of cold temperature storage (0-10 ºC) or ethylene (100 ppm) to induce pear ripening (Makkumrai et al., 2014). Early harvest pears that may not be fully mature need to be conditioned with ethylene or placed in cold storage to ensure they ripen correctly. Late harvest pears, which are more mature,

or pears stored at low temperature (4 ºC) for three weeks, do not require ethylene conditioning (Kader & Mitcham, 2002).

As summer pears, California 'Bartlett' pears are typically harvested in July and August (Villalobos-Acuña et al.). The pears are usually hand-packed into bags then placed into field bins designed to avoid bruising. The bins are transported to a packing facility, where they are washed with 100-150 ppm chlorinated water (pH 6-7) in a float tank and then sorted to eliminate defective or undersized pears (OSU, 2020). In addition to washing and before sorting, pears are treated with fungicides such as Merivon® (4-5.5 fl oz/A) or Pristine® (14.5-18.5 oz/A) to prevent the growth of blue and gray mold (OSU, 2020). After sorting, pears are packed into cartons and separated into those for marketing and those for processed products (Agar & Mitcham, 2000).

Pears are then forced-air cooled to 1-2 ºC (40 ºF) to remove residual warm temperatures and placed into cold storage (-1 ºC, 30 ºF) until distribution. In California, 'Bartlett' pears can be stored in air for two to three months (Mielke & Drake, 2004). 'Bartlett' pears placed in long-term storage for up to six months should be under an optimum controlled atmosphere (CA) of 1-3% O_2 and 0-3% CO_2 (Mitcham, 1996), even though CA is not utilized in California at this time. For early and mid-season 'Bartlett' pears, the optimum CA is 1.5-2% O_2 and 1-5% CO_2. Late-season pears should be kept below 1% CO_2 to avoid browning of the core and flesh due to an increased sensitivity to CO_2 (Mitcham, 1996). Conditioning with 1-5% CO_2 can slow the rate of respiration, ethylene (C_2H_4) production, softening, color change from green to yellow, and can inhibit the development of physiological disorders. Although a complete understanding of why 1-5% CO_2 can have these effects is not entirely known, it is suggested that CO_2 competes with ethylene at the ethylene binding protein of the receptor site that initiates ethylene biosynthesis, thereby inhibiting ethylene production (Hans, Els, & Herman, 2003).

2.3 Ripening in ‘Bartlett’ pears

Ripening in pears is triggered and mediated by ethylene (Busatto et al., 2019). Since ‘Bartlett’ pears are climacteric fruit, respiration rate and ethylene production will increase as they ripen (Nham et al., 2015). However, early-season ‘Bartlett’ pears resist ripening after harvesting due to low ethylene production. ‘Bartlett’ pears require either low-temperature storage at -1-0 ºC (30-32 ºF) with relative humidity at 90-95% for three weeks, exogenous ethylene treatment of 100 ppm C_2H_4 for 24-48 h at 20 to 25 ºC, or a combination of these two factors to induce ripening (Makkumrai et al., 2014; Zlatić et al., 2016). The biochemical reasoning behind a postharvest resistance to ripen and the use of conditioning to induce ripening are not well described or understood (Villalobos-Acuña & Mitcham, 2008).

Ethylene (C_2H_4) affects the growth and development of pears and stimulates their ripening, respiration, pigment synthesis, and senescence. Ethylene synthesis and action are metabolic pathways that require O_2 and are sensitive to high concentrations of CO_2 (Saltveit, 1999). A burst of ethylene after harvest promotes the ripening process in pears. Negative feedback inhibition changes to positive feedback promotion during pear ripening, and ethylene synthesis is stimulated. The promoting effect of ethylene treatment on ripening eventually plateaus when its internal concentration has increased to saturation levels as the climacteric peak is reached (Saltveit, 1999).

The ripening of pears involves a shift from auto-inhibitory ethylene (System 1) to autocatalytic ethylene (System 2) that regulates the overall ripening process. The functions of system 1 occur during growth, development, and responses to stresses such as chilling. In system 1, ethylene is autoinhibitory, and exogenous ethylene inhibits internal ethylene production while ethylene inhibitors can stimulate it (Barry & Giovannoni, 2007). The functions of system 2 operate during ripening and senescence. In contrast to system 1, ethylene stimulates its own production in

system 2, making it autocatalytic, and the action of an ethylene inhibitor inhibits ethylene production (Barry & Giovannoni, 2007).

As 'Bartlett' pears ripen to a flesh firmness of 2-4 lb_f, they soften and exhibit a strong, pleasant aroma; a sweet taste; and a juicy, buttery texture (Mitcham, 1996). These quality changes are mediated by ethylene during ripening that induces the gene expression of aroma volatile production and enzymes critical for cell wall breakdown. Low ethylene production results in impaired ripening, leading to poor eating quality because pears develop low concentrations of volatile compounds and a coarse texture. Impaired ripening can occur when pears remain on the tree during ripening or are left to slowly ripen after harvest at 20 ºC (Makkumrai et al., 2014).

2.4 Ethylene production and biosynthesis

Two enzymes that have a crucial role in ethylene biosynthesis are 1-aminocyclopropane-1-carboxylate synthase (ACS) and 1-aminocyclopropane-1-carboxylate oxidase (ACO). The starting point for ethylene biosynthesis is the amino acid methionine (**Figure 2**). The addition of adenine converts methionine to S-adenosyl-L-methionine (SAM). This step is followed by the conversion of SAM to 1-amino-cyclo-propane carboxylic acid (ACC) as catalyzed by ACC synthase or ACS (Bulens et al., 2011). This conversion of SAM to ACC is often the rate-limiting step for ethylene synthesis. Lastly, ACC is oxidized by ACO to ethylene (Saltveit, 1999). Because the oxidation of ACC to ethylene requires oxygen, the reaction is regulated by either low levels (1-2% O_2) of oxygen or high levels (5-20% CO_2) of carbon dioxide (Pedreschi et al., 2008). As such, low oxygen levels or high levels of CO_2 will decrease ethylene production (Paul & Pandey, 2014).

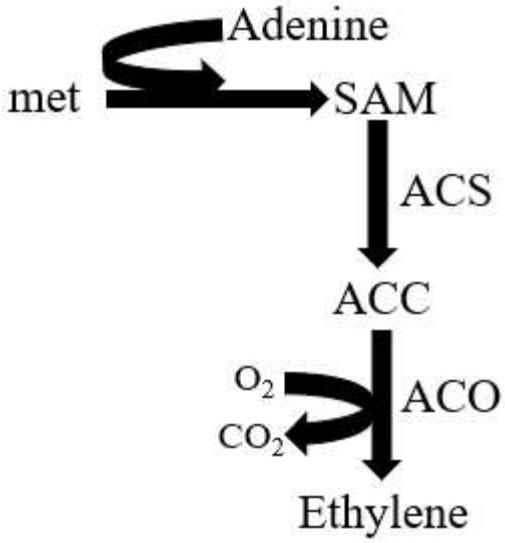

Figure 2. Ethylene biosynthesis from methionine (Adapted from Saltveit, 1999).

2.5 Postharvest defects in 'Bartlett' pears

The primary postharvest defects in 'Bartlett' pears are senescent scald, core breakdown, and superficial scald with internal browning and watery breakdown also reported (Crisosto, 2020).

2.5.1 Senescent scald in 'Bartlett' pears

Senescent scald appears as a brown to black discoloration of the skin (UCD, 2020) on abnormally ripened pears and is etiologically different from superficial scald in that superficial scald develops during the warming period after removal from storage as irregular brown areas on the peel (Lum et al., 2017). Although restricted to the skin, this discoloration can proceed into the flesh. This defect can develop on pears that have been stored beyond their postharvest life of 6-8 months. Senescent scald begins as darkened spots around the calyx in a uniform pattern then spreads upward in the fruit (Whitaker et al., 2009). As a result, senescent scald can lead to fruit loss during extended storage or transportation.

The metabolic processes that result in senescent scald are not fully understood at this time but, it is hypothesized that as the fruit dies, the availability of energy molecules such as ATP and NADPH decreases. This, results in decreased levels of antioxidants such as ascorbate and glutathione in stored pears (Flaherty et al., 2018). Without these antioxidants, the fruit is left without the mechanisms to repair the damage by reactive oxygen species that result from the metabolic shift from aerobic to anaerobic pathways (Lum et al., 2017). Senescent scald is associated with ethylene production in pears during storage (Lum et al., 2017). **Figure 3** illustrates the posited mechanism of senescent scald development in 'Bartlett' pears.

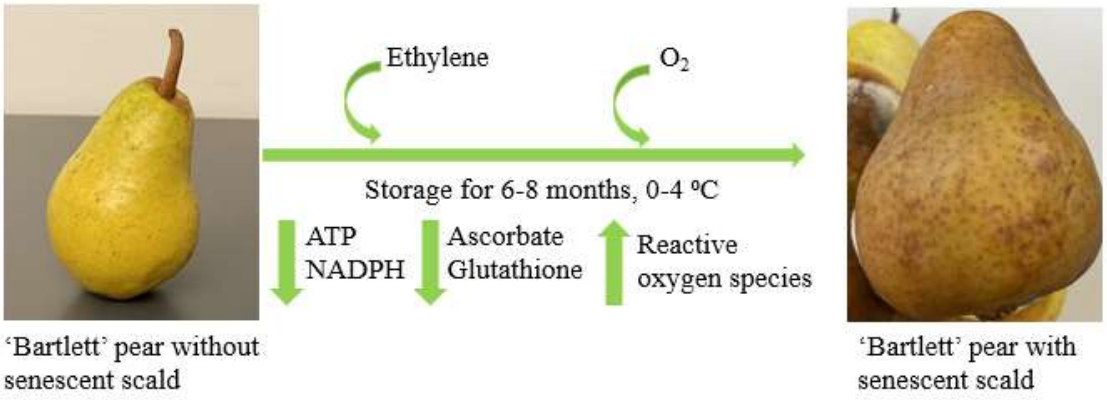

Figure 3. Proposed mechanism for the development of senescent scald in 'Bartlett' pears (Adapted from Flaherty et al. 2018 and Lum et al. 2017.

2.5.2 Control of senescent scald in 'Bartlett' pears

Zhao et al. (2018) found that 1-MCP inhibited senescent scald while prolonging the shelf-life of the treated pears by decreasing ethylene production. Lum et al. (2017) also found that CA as low as 2.5 kPa O_2 effectively prevented senescent scald, but even higher inhibition was achieved through a combination of CA and 1-MCP (300 nL L^{-1} 1-MCP; 2.5 kPa O_2 and 2 kPa CO_2).

2.6 Core breakdown, watery breakdown, and associated internal browning in 'Bartlett' pears

Core breakdown results from senescence that develops into a softening of the core and surrounding tissues with a brown appearance making the pear unfit to consume (UCD, 1971). With core breakdown, there is a separation of watery affected tissue from healthy tissue in these defects. Tissue with a watery breakdown is not discolored at first but will brown over time, making the defects indistinguishable. Browning of internal tissue develops when membrane damage of the cells that comprise the tissue occurs, allowing polyphenols and polyphenol oxidases (PPOs) entry into the cytoplasm (Deuchande et al., 2016).

In the cytoplasm, these polyphenols and PPOs oxidize phenols that result in the formation of o-quinones that polymerize into the browning melanin compounds (Deuchande et al., 2016). **Figure 4** depicts the mechanism of internal browning in core breakdown in 'Bartlett pears.

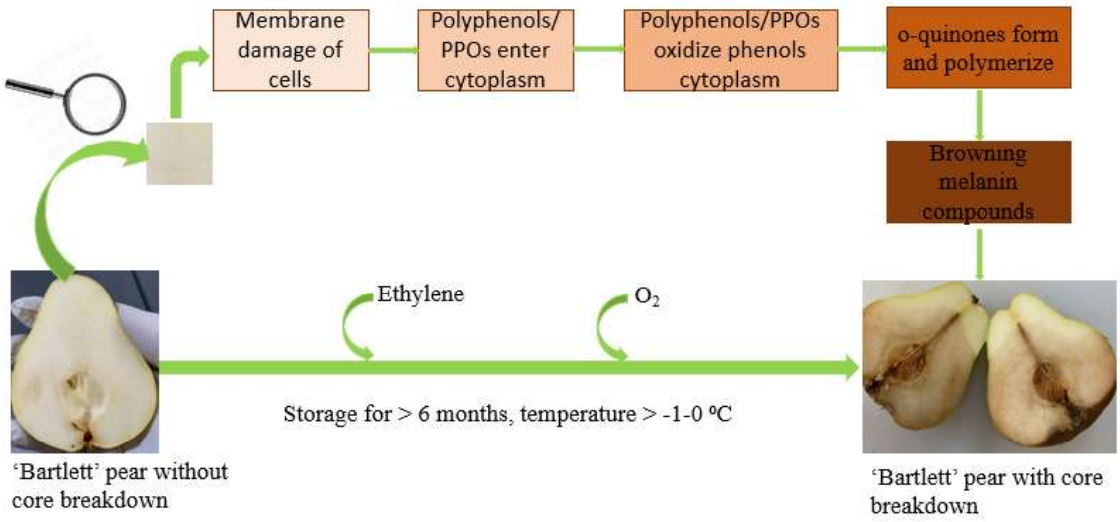

Figure 4. Mechanism of internal browning in core breakdown in 'Bartlett' pear. Adapted from Deuchande et al. (2016).

Watery breakdown is not a senescent disorder, but it is a disorder caused by improper storage or ripening conditions caused by a slow cooling rate after harvest or holding at a higher temperature than recommended for 'Bartlett' pears (UCD, 1971). Core breakdown is a senescent disorder and is also caused when pears are not cooled to 1-2 ºC after harvest and placed in higher temperatures than recommended (-1-0 ºC) or are placed in storage longer than six months (Crisosto and Mitcham, 2020).

Pears may be stored under CA to mitigate quality degradation. However, if the partial pressure of oxygen is too low (<1% O_2) and carbon dioxide is too high (>3% CO_2), then aerobic metabolism can shift to anaerobic metabolism resulting in the core breakdown (Pedreschi et al., 2007). High CO_2 partial pressure during storage can cause localized hypoxic conditions in the core leading to oxidative stress and metabolic shifts towards energetically less efficient fermentation pathways. This shift leaves the pears unable to repair damage caused by reactive oxygen species to the cellular membrane. As this damage to the membrane occurs, enzymatic oxidation of

phenolic substrates occurs, leading to browning. Eventually, cell death and water loss to the environment follows, and cavities appear in the core (Pedreschi et al., 2009).

Fermentation products (ethanol and acetaldehyde) and related enzymes (pyruvate dehydrogenase and alcohol dehydrogenase) generally increase in pears under long-term CA storage as a result of a switch from aerobic to anaerobic respiration (Deuchande et al., 2016). Damaged fruits exhibit a substantial increase in these compounds during storage. Higher concentrations of CO_2 and the accumulation of acetaldehyde are attributed to stored fruit that has displayed core breakdown (Pintó, Lentheric, Vendrell, & Larrigaudière, 2001).

2.6.1 Control of core breakdown in 'Bartlett' pears

Preharvest and postharvest treatment of 1-MCP mitigates core breakdown in 'Bartlett' pears during storage by inhibiting ethylene production and metabolic activity associated with senescence-related disorders (Pareek, 2019). Villalobos-Acuña et al. (2011) found that postharvest treatment of 0.3 $\mu L\ L^{-1}$ 1-MCP at 20 ºC for 12 h effectively controlled core breakdown in 'Bartlett' pears.

Ju et al. (2001) found that dipping pears in either corn or soybean 5-10% oil emulsions for 3 min followed by storage at 0 ºC for 15 weeks can control core breakdown in preclimacteric 'Bartlett' pears. The oil emulsions applied to the surface of the fruits sealed sites of gas diffusion by forming a thin layer, thereby modifying the internal atmosphere of the fruit, and altering the permeability of oxygen and carbon dioxide. This alteration to oxygen and carbon dioxide permeability prevented fermentation reactions related to core breakdown (Ju et al., 2001).

2.7 Superficial scald in 'Bartlett' pears

Characterized as areas of brown or black patches on the skin of the fruit (Crisosto, 2020), superficial scald develops after removal from extended storage under low temperature (-1-0 ºC) and during a warming period of five to seven days. Damage and death of surface layer cells of the pears results in the discolored regions of superficial scald (Lurie & Watkins, 2012). This defect develops because of a high accumulation of α-farnesene and its autoxidation products, conjugated trienols (CTols), that lead to cell damage and death (Whitaker et al., 2009). Ethylene production regulates the synthesis and subsequent accumulation of α-farnesene in the fruit peel (**Figure 5**).

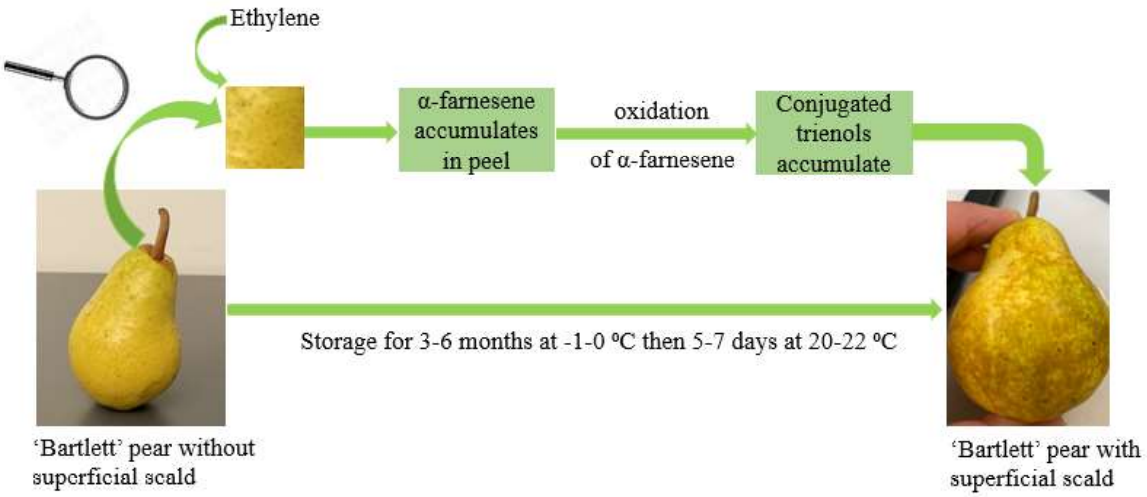

Figure 5. Development of superficial scald on 'Bartlett' pear. Adapted from Lurie and Watkins (2012) and Whitaker et al. (2009).

As α-farnesene accumulates, so do CTols, which induce the necrosis and cell damage of hypodermal cortical tissue responsible for superficial scald (Lurie & Watkins, 2012). Low-temperature storage of pears susceptible to superficial scald synthesizes α-farnesene at a high rate leading to its accumulation in the fruit's skin during the initial two to three months of cold storage. After two to three months, α-farnesene concentration declines while the concentration of CTols increases (Gapper, Bai, & Whitaker, 2006).

The enzyme α-farnesene synthase is critical for the biosynthesis of α-farnesene. Pechous and Whitaker (2004) found that expression of *AFS1*, the gene responsible for encoding α-farnesene synthase in apples, is promoted by ethylene. *AFS1* was strongly associated with the buildup of α-farnesene, leading to the development of superficial scald in the peel of apples such as 'Law Rome,' and 'Idared' apples (Pechous, Watkins, & Whitaker, 2005) and 'Granny Smith' apples (Melo et al., 2021). Therefore, inhibiting ethylene synthesis will inhibit the gene expression of *AFS1*, the production of α-farnesene, and its subsequent accumulation. Superficial scald typically develops after the fruit is removed from storage to warmer temperatures (20-22 ºC). However, it may occur over extended storage of up to 6 months (Lurie & Watkins, 2012).

2.7.1 Control of superficial scald in 'Bartlett' pears

There are several strategies to inhibit the development and incidence of superficial scald in 'Bartlett' pears such as the use of 1-methylcyclopropene (1-MCP), ethoxyquin, diphenylamine (DPA), and controlled atmosphere (CA).

2.7.2 Use of 1-Methylcyclopropane to control superficial scald

Under its brand name, SmartFresh™, 1-MCP has been commercially applied to fruit and vegetable industries to delay the ripening process. In 2002, the Environmental Protection Agency (EPA) established a regulation providing a tolerance exemption of 1-MCP for its residues in or on fruits and vegetables when used as a postharvest treatment (EPA, 2016). As an ethylene inhibitor, 1-MCP can block α-farnesene synthesis by inhibiting ethylene synthesis and its gene expression in 'Bartlett' pears. 1-MCP irreversibly binds to ethylene receptor sites, thereby inhibiting ripening and senescence responses dependent on ethylene production (Zhao et al., 2018). As a result, the buildup of α-farnesene and CTols decreases in the peel, thus preventing superficial scald. Pechous,

Watkins, and Whitaker (2005) concluded that apples treated with 1-MCP suppressed *AFS1* expression resulting in an apparent inhibition of α-farnesene production.

Villalobos-Acuña et al. (2011) found that 'Bartlett' pears treated with 0.3 μL L^{-1} 1-MCP for 12 h at 20 ºC decreased the rate of superficial scald. Isidoro and Almeida (2006) found that an application of 1-MCP at 0.1 μL L^{-1} decreased superficial scald with no effect on ripening with 'Rocha' pears stored for 4 months at 0 ºC. However, in some cases, superficial scald may be prevented at the cost of the ability of the fruit to ripen properly. As a result of 1-MCP treatment, the ability of pears to ripen normally after storage may be unpredictable (Argenta, Mattheis, Fan, & Amarante, 2016). California pear growers have discontinued using 1-MCP as a postharvest treatment because pears may take as long as three weeks to ripen (FGN, 2020).

2.7.3 Use of antioxidants to control superficial scald

Superficial scald results from the damage imparted on the cellular tissue of the peel by CTols, the oxidation products of α-farnesene. Synthetic antioxidants such as 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (ethoxyquin) and diphenylamine (DPA) can control superficial scald by inhibiting the accumulation of CTols (Yu & Wang, 2017). Ethoxyquin can be applied to pears at 2.4 g L^{-1} up to seven days after harvest (Lurie & Watkins, 2012). Ethoxyquin can be used as a drench, line spray, or used in paper wraps (Hansen & Mellenthin, 1979). 'Anjou' pears treated with 1000 ppm ethoxyquin effectively controlled superficial scald after 1-month storage in air following five months storage under CA (1.0 ± 0.1% O_2 and 1.0 ± 0.1% CO_2) (Mielke & Drake, 2004). 'Anjou' pears treated with 1000 μL L^{-1} of ethoxyquin did not show evidence of superficial scald for three months after storage (Bai et al., 2009). Bai et al. (2009) also found that treatment of 25 nL L^{-1} 1-MCP in addition to 1000 μL L^{-1} of ethoxyquin controlled superficial scald up to

five months after storage. Commercial application of 1000 and 2000 μL L^{-1} DPA to 'Dangshansuli' pears inhibited α-farnesene oxidation and decreased the CTol content and polyphenol oxidase enzyme activity after 210 days in cold storage (De-ying, 2011). Isidoro and Almeida (2006) found the application of 636 mg L^{-1} to 'Rocha' pears after harvest decreased superficial scald without any lingering effect on ripening capacity after 4 months in storage.

Although ethoxyquin mostly remains in the fruit's peel when applied, it can penetrate deeper into the fruit (López & Riba, 1999). The advantages of using ethoxyquin and DPA are their high antioxidant capacity at a low production cost. However, there are concerns regarding the application of ethoxyquin and DPA as anti-scalding agents. In vitro studies suggest that ethoxyquin could result in adverse health consequences by causing chromosomal mutations or aberrations in human lymphocytes (white blood cells). These white blood cells aid the immune response system (Rodríguez-Gómez, Vandeput, Zafra-Gómez, & Kauffmann, 2018). Beginning in 1962, the commercial use of DPA in the United States spread due to its low production cost, a simple application, and presumed low toxicity. How long DPA and its metabolite residues remain in the pulp of fruit depends on the type and variety. In 'Red Delicious' apples, a residue of 17 mg/kg resulted after treated apples were stored for 40 weeks. However, the detection of nitrosamines (0.05 mg/kg), the carcinogenic residues generated by DPA combined with nitrogen, in crops have led to concerns over the use of DPA in the United States and its eventual disuse as an anti-scalding agent in the European Union (Dias et al., 2020; European Food Safety, 2011).

2.7.4 Use of controlled atmosphere to control superficial scald

Controlled atmosphere (CA) can maintain quality over extended periods, thereby prolonging shelf-life (Hansen & Mellenthin, 1979). Controlled atmosphere can also delay and decrease the severity of superficial scald in 'Bartlett' pears with O_2 (1.5 kPa) and CO_2 (< 1 kPa) for 5 months at -1.1 ºC (Mitcham, 1996; Zhi, Dong, & Wang, 2019). Converting ACC to ethylene by ACO requires oxygen; therefore, if oxygen exposure levels are kept low (1-2% O_2), then ethylene production will be decreased (Pedreschi et al., 2008). Drake, Gix, and Coureau (2001) found that 'Anjou' pears placed under CA of 2% O_2 for 90 days and then regular air atmosphere (RA) for 30 days had 16.7 % less occurrence of superficial scald after ripening than untreated pears (Drake et al., 2001). However, the use of CA for long-term storage of pears needs equipment and sensors that require a capital investment and is expensive to operate (Falagán & Terry, 2018).

Factors that influence the effectiveness of CA in controlling superficial scald are fruit cultivar, storage temperature, maturity, and pre-storage treatment. If not used appropriately, CA can induce the development of off-odors and off-flavors in the fruit. To successfully use CA, a complete understanding of the physiology of the harvested fruit and how to adapt CA technology to the physiology need to be understood by the user. CA as a postharvest treatment is not used in California because it is expensive to operate, and a specialized knowledge of the technology and harvested fruit is required (Falagán & Terry, 2018; McClain, 2019).

2.8 Using irradiation as an alternative to inhibit postharvest defects in 'Bartlett' pears

Ionizing irradiation may offer an alternative to mitigating the primary postharvest defects associated with 'Bartlett' pears without leaving chemical residues or using CA. Irradiation is

increasingly used as a phytosanitary treatment for papayas, mangoes, apples, and pears. Irradiation can affect their ripening and retard certain fruit defects as described in sections 2.6.3, 2.6.4, and 2.7 (Hallman, 2011).

2.8.1 Types of irradiation

Irradiation, or ionizing radiation, has three modalities. It can be an emission of waves from x-rays with a maximum energy level of 7.5 MeV, or it can be in the form of moving particles like an electron beam (e-beam) with a maximum energy level of 10 MeV. Ionizing radiation can also be gamma rays from isotopes cobalt-60 (with levels of 1.17 and 1.33 MeV) or cesium-137 (with 0.662 MeV). The energy of these types of radiation is enough to remove electrons from atoms or molecules, classifying it as ionizing radiation, but not enough to induce radioactivity when applied as food irradiation (Codex, 2003; FDA, 2019). The energy absorbed by the irradiated product quantifies the dose of irradiation (GMA, 2009).

E-beams are produced by electron accelerators that accelerate electrons to the speed of light. Electrons can break molecular or atomic bonds at this speed, thus releasing free electrons and ions that can then react with other particles or charged molecules and atoms, creating secondary ions in the process. The excited electrons are then targeted on the food through a titanium window by a scan horn after being sized and oriented (Pillai, Shayanfar, & Bolumar, 2015). E-beams are then targeted onto a metal filter where some of the energy is converted to x-rays while the rest is absorbed by the metal filter (Bruhn, 2017). As the high-speed electrons travel through the metal filter, they will slow down due to interactions with electrons they encounter in the metal. The resultant radiation emitted by the charged electron, bremsstrahlung radiation, is in the form of x-rays (Lloyd-Jones, 2016).

E-beams and x-rays differ in that e-beams only have a maximum penetration depth of 8 cm. This penetration depth is not enough to extend shelf-life through the reduction or elimination of microorganisms and insects. Unlike e-beams, x-rays have a high depth of penetration of up to several feet. However, x-ray irradiation is an expensive operation because of the inefficiency of converting electricity into x-rays, which, at most, is 14% with the rest of the converted electricity given off as heat (Hallman, 2011; WHO, 1988). Like x-rays, gamma rays from isotopes Cobalt-60 and Cesium-137 can penetrate up to several feet in food (WHO, 1988).

The actual penetration depth is dependent on the density of the food product. Gamma irradiation has great penetrating power because it is generated by photons that do not have mass given off by isotopes (Co-60 and Cs-137). For e-beam and x-rays, the penetration power depends on the accelerator. Photons in x-rays are like gamma irradiation because of their great penetrating power (Pillai, 2016).

Radiation dose determines the extent of primary and secondary effects of irradiation. Usually, there is a linear relationship between dose and radiolytic product formation in food irradiation. For example, increasing the dose by a factor of two will increase the number of radiolytic products by a factor of two. Yet, if a radiolytic product is formed at a low dose of <1.0 kGy, the radiolytic product may be destroyed by radiation, making the relationship between dose and product formation nonlinear. There is also the possibility that a scavenger may be present in the irradiated system that could inhibit product formation. However, at a high dose (>1.0 kGy), consumption of the scavenger can occur, and product formation will increase (Diehl, 1999).

The dose rate is the quantity of radiation absorbed per unit time. There are differences in dose rates for each of the three modalities. **Table 2** indicates the different dose rates of each

modality at different energy levels (Pillai, 2016). Radiolytic products formed in a sample are the same, whether from a small or large gamma source because the dose rate is low. Conversely, because the dose rate for e-beam and x-ray is much higher than gamma, radiolytic products that form in such a short time have a higher probability of reacting with each other than with molecules of the irradiated system (Diehl, 1999).

Table 2. Irradiation modality and associated dose rates (Diehl, 1999; Pillai, 2016)

Source	Energy, MeV	Dose rate, Gy/s
Gamma	1.59	~0.06-0.12
X-ray	5	~100
	0.1	~0.01
Electron beam	10	~3,000
	8.5	~3,000

2.8.2 Phytosanitary irradiation

Fresh fruits and vegetables can be vehicles for pests. To prevent pests from being introduced into new areas or regions, resulting in infestations and economic loss, exporting countries believed to be pest infestation areas need to establish phytosanitary security of their fresh fruit and vegetable exports. The commercial use of ionizing irradiation as a phytosanitary treatment of fresh fruits and vegetables against insect pests, such as fruit flies and moths, has been adopted in exporting countries like Thailand, Mexico, and Vietnam. While fumigants are becoming progressively restricted and expensive, these countries are expanding their use of phytosanitary irradiation to bypass trade barriers and broaden their exports into new markets (Hallman, 2011; Hallman & Blackburn, 2016).

When applied to fruits and vegetables as a phytosanitary treatment, irradiation disrupts organic molecules such as DNA within infestation pests by breaking chemical bonds in the DNA structure and other biomolecules such as lipids and proteins for normal cellular function (McHugh & Liang, 2019). Sufficient damage to these cellular components can overcome the repair mechanisms resulting in cell death. The radiolytic compounds produced by irradiation result in cellular membrane lipid oxidation, leading to cell death (Prakash & Ornelas-Paz, 2019). Cell division is easily affected by reactive oxygen species produced by irradiation causing adult insects to cease reproduction and feeding. The goal of phytosanitation is not acute mortality, but to prevent infestation pests from maturing further or from reproducing (Hallman, 2011; Hallman, 2013). Except for pupae and adults, a generic dose of 400 Gy is used for all insects classified in order Lepidoptera while a generic dose of 150 Gy is used for any tephritid fruit fly (Hallman & Blackburn, 2016; Follett & Wall, 2013). The multiple benefits of using irradiation as a phytosanitary treatment are illustrated in **Table 3**.

Table 3. Benefits of irradiation as a postharvest treatment of 'Bartlett' pears (McHugh & Liang, 2019)

Technical	Economical	Consumer benefits	Regulatory
Temperature and chemical independent; Safe; energy efficient	Global food irradiation market value as of 2017 was $200 million	Increased quality and prolonged shelf-life of fresh Bartlett' pears	Microbial decontamination Approved as a quarantine treatment for fresh produce and vegetables

2.8.3 Effect of irradiation on ripening

Irradiation can affect fruit ripening by impacting ethylene production. In climacteric fruit such as papayas, mangoes, apples, and pears, irradiation can delay or inhibit ethylene production, and thus, delay ripening.

2.8.4 Effect of irradiation on pear ripening

Maxie, Sommer, Muller, and Rae (1966) found that doses of 3 kGy or more inhibited ripening in 'Bartlett' pears. Pears treated with doses between 3-4 kGy failed to ripen even when exposed to ethylene for eight days at 20 ºC. Maxie et al. (1966) concluded that the effect of gamma radiation on 'Bartlett' pears was a combined effect of inhibition of ethylene production with decreased sensitivity of pears to the ripening action of ethylene. Maxie et al. (1966) also found that ripening was only inhibited by irradiation in pre-climacteric fruit (Maxie et al., 1966). In another study, pears treated with 2-3 kGy delayed ripening for two to three days by suppressing ethylene production (Sattar, Ali, Khan, & Muhammed, 1971).

Abolhassani et al. (2013) findings indicated that irradiation at dose levels of 400 and 600 Gy for early harvest and late harvest 'Bartlett' pears delayed ripening by one to two days. Abolhassani et al. (2013) suggested that the delay in ripening could increase shelf-life by delaying softening or senescence. Abolhassani et al. (2013) also observed that the color of irradiated early harvest pears was greener than untreated control pears. Incomplete chlorophyll degradation by the chlorophyllase enzyme was offered as the rationale behind this observation. Sea et al. (2015) found that 'Bartlett' pears subjected to x-ray irradiation at a dose level of 400 Gy had a longer shelf-life than the control pears. A longer shelf-life was attributed to the effect that irradiation may have had on protopectinase and pectin methylesterase (Sea et al., 2015). These enzymes catalyze reactions

that convert insoluble pectin fractions to soluble forms, decreasing firmness during ripening. Sea et al. (2015) suggested that irradiation inhibited protopectinase and pectin methylesterase activity.

2.8.5 Relationship between irradiation and fruit maturity

Besides fruit species and cultivars, how low dose irradiation alters fruit ripening also depends on the maturity stage of the fruit at the time of treatment (Barkai-Golan & Follett, 2017). Mature papaya fruit irradiated with 500 Gy and ripened at 22 ºC and 90 % relative humidity resulted in a two-day delay before ripening began with all the characteristic changes in firmness, color change, and sugar content to occur normally (D'Innocenzo & Lajolo, 2001). D'Innocenzo and Lajolo (2001) observed a continuous increase in enzyme activities of polygalacturonase, pectin methylesterase, and β-galactosidase in treated papayas until the end of the climacteric period at ten days. D'Innocenzo and Lajolo (2001) proposed that an irradiation repair mechanism may allow the restoration of protein synthesis for fruit treated before the climacteric stage that allowed for the enzymatic activity to progress as it did. Singh and Pal (2009) observed that mature light green guavas ('Lucknow-49' and 'Allahabad Safeda' cultivars) exposed to 0.25 kGy of gamma irradiation during the preclimacteric stage exhibited a delay in ripening. They attributed this finding to the inhibitory effect that irradiation has on ethylene production, implying that the ripening enzymes were altered by irradiation through their gene expression (Singh and Pal, 2009).

The effects of electron-beam radiation at a dose range of 1.0-3.1 kGy on green mangoes before and during storage delayed ripening (Reyes & Cisneros-Zevallos, 2007). Reyes and Cisneros-Zevallos (2007) suggested that the reactive oxygen species produced by irradiation might have inhibited key enzymes such as polyphenol oxidase, peroxidase, and catalase associated with ripening by altering their gene expression (Frylinck, Dubery, & Schabort, 1987). Mahto and Das

(2013) irradiated two cultivars of mature green mangoes and found that doses of 0.3-0.7 kGy (for 'Dushehri') and 0.5-0.7 kGy (for 'Fazli') of gamma radiation delayed ripening for three days. A mechanism on how radiation delayed ripening was not offered, but Mahto and Das (2013) suggested that the reactive oxygen species produced by irradiation could alter the gene expression of ripening enzymes, causing a shift in the endogenous ethylene peak (Mahto & Das, 2013).

2.8.6 Effect of irradiation on mold

Some of the significant postharvest pathogens of pears are *Penicillium expansum* and *Botrytis cinerea* (**Figure 6**; Mitcham, 1996). Growth of these postharvest pathogens occurs when they enter and colonize wounds and punctures on the surface of injured pears (Mitcham, 1996). Geweely and Nawar (2006) found that one day of treatment of 1 kGy completely inhibited spore germination of both molds.

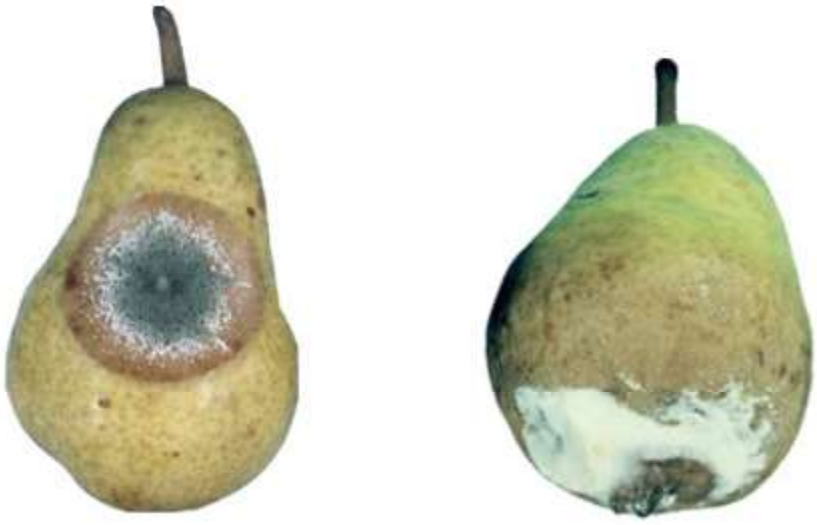

Figure 6. Penicillium expansum (left) and Botrytis cinerea (right) on non-irradiated 'Bartlett' pears (Crisosto, 2020).

Geweely and Nawar (2006) concluded that radiosensitivity to gamma radiation was due to the thin cell walls of the two fungi. Because of these thin cell walls, gamma radiation could directly damage

cellular DNA. There was also an indirect effect on fungi cells resulting from the radiolysis of cellular water and the production of reactive oxygen species, peroxides, and free radicals that caused breakages within the DNA strand (Geweely & Nawar, 2006). Jeong, Jeong, and Park (2017) found that gamma irradiation with dose levels of 100 and 200 Gy decreased the incidence of *P. expansum* infection by about 60%. Jeong, Jeong, and Park (2017) showed that these dose levels decreased infection by enhancing the activity of enzymes related to disease resistance such as β-1, 3-glucanase, phenylalanine ammonia lyase (PAL), peroxidase, and polyphenol oxidase, thereby inhibiting fungal infection. PAL affects the synthesis of compounds like polyphenols and lignin that build up in plants in response to pathogen infection. Oxidoreductases like peroxidase (POD) are involved in phenol oxidation and lignification in cell walls. Lastly, PPO catalyzes the oxidation of polyphenols that produce antimicrobial compounds as a further response to pathogen infection (Jeong, Jeong, & Park, 2017).

2.9 Effect of irradiation on postharvest defects

Irradiation can inhibit superficial scald in fruit but can increase internal browning as summarized in the following sections. However, there is no literature on the effect of irradiation on other postharvest defects in pears, such as core breakdown and senescent scald.

2.9.1 Effect of irradiation on superficial scald

Massey, Parsons, and Smock (1964) evaluated the effect of gamma irradiation at 0.5 and 1.0 kGy on 'McIntosh,' 'Cortland,' and 'Rome Beauty' apples. All three apple varieties experienced less storage scald as dose levels increased (Massey, Parsons, & Smock, 1964). Al-Bachir (1999) also reported that 'Golden Delicious' apples irradiated with dose levels up to 1.5

kGy prevented scald formation. Drake, Sanderson, and Neven (1999) found that scald development increased in 'Anjou' pears with doses 0.60 kGy or higher, but not in 'Bosc' pears, indicating that scald formation and its mitigation by irradiation may depend on the pear variety. 'Gala' apples treated with gamma radiation up to 1.32 kGy almost eliminated scald after storage for 8 weeks at 0 ºC plus 7 days at 20 ºC (Fan & Mattheis, 2001). Fan and Mattheis (2001) suggested that a dose-dependent treatment of irradiation combined with time-dependent storage at 0 ºC may synergistically decrease scald on 'Gala' apples.

Melo et al. (2021) found that 'Granny Smith' apples treated with 1.0 kGy did not exhibit superficial scald after 180 days at 0-1 ºC followed by storage at room temperature for 7 days, while fruit treated with 310 Gy had an 18% occurrence of superficial scald. In contrast to treated apples, 38-42% of the control fruit exhibited scald after 90-days of observation. Melo et al. (2021) also found that ethylene production of irradiated fruit was lower than non-irradiated fruit at both 90 and 180 days. A similar trend was also observed with the α-farnesene concentration in irradiated fruit compared to the control fruit. After 90 days, the α-farnesene concentration was decreased by more than 50% in irradiated fruit compared to control fruit (Melo et al., 2021). Melo et al. (2021) and Nyakundi, Prakash, Atamian, and Olabode (2019) observed that the low incidence of superficial scald and α-farnesene accumulation after irradiation treatment of the fruit was correlated with a decrease in ethylene production. Nyakundi et al. (2019) found that irradiation inhibited the expression of α-farnesene synthase, the final enzyme in the α-farnesene pathway, after 97 days. Irradiation also inhibited ACC oxidase activity and decreased ethylene production, which correlated with decreased superficial scald development.

2.9.2 Effect of irradiation on internal browning

Fan and Mattheis (2001), Reyes and Cisneros-Zevallos (2007), and Jenjob et al. (2017) observed irradiation-induced internal browning of fruit. Fan and Mattheis (2001) found that the incidence of internal browning of irradiated 'Gala' apples increased from 0.44-1.32 kGy but did not specifically offer a rationale behind this observation. Reyes and Cisneros-Zevallos (2007) observed internal browning in mangoes irradiated with doses ≥ 1.5 kGy. Reyes and Cisneros-Zevallos (2007) suggested that PAL activity produced phenolic compounds that resulted in internal browning from their accumulation. (Jenjob et al., 2017) found that pineapple irradiated with 400-600 Gy induced internal browning. Jenjob et al. (2017) attributed this development of internal browning to an increase in PPO and PAL activity associated with internal browning (Jenjob et al., 2017).

Melo et al. (2021) observed that 43% of 'Granny Smith' apples irradiated with 1000 Gy developed internal browning after 90 days of storage at 0 ºC plus seven days at room temperature (20 ºC), while fruit treated with 310 Gy showed no incidence of internal browning. However, after 180 days plus seven days, 19.4% of apples treated with 310 Gy did show some internal browning. This finding was attributed to the irradiation-induced formation of reactive oxygen species that stimulated PAL activity, allowing for phenolic compounds to accumulate, resulting in internal browning (Melo et al., 2021). Internal browning was not observed in apples treated with 310 Gy. At a lower dose level, fewer ROS were likely quenched by antioxidant and enzymatic systems. At higher doses, the high rate of ROS overwhelmed these systems allowing for internal browning to develop (Melo et al, 2021). **Figure 7** describes a mechanism of how irradiation inhibits postharvest defects in 'Bartlett' pears.

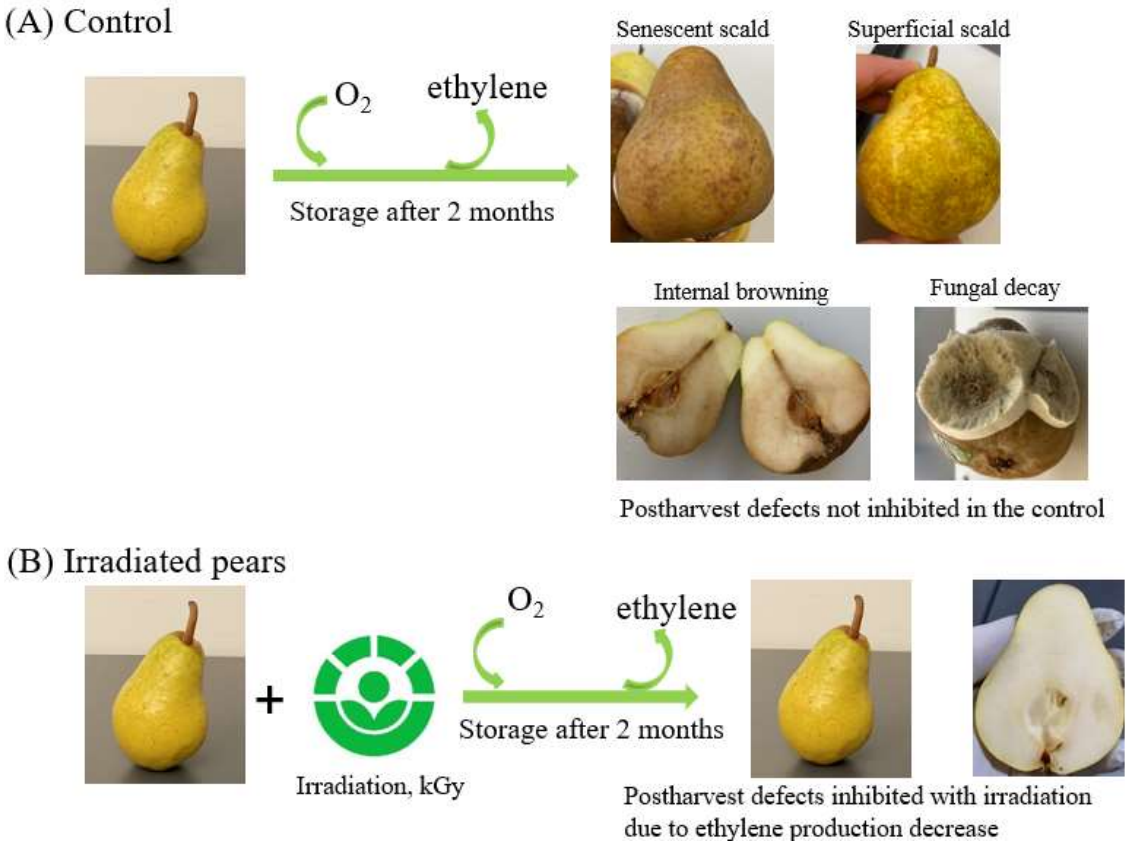

Figure 7. Mechanism of irradiation inhibiting postharvest defects. Without irradiation treatment (A), ethylene production will not decrease, and pears will develop postharvest defects. In contrast, ethylene production will be decreased, and postharvest defects in pears will be inhibited with irradiation treatment (B).

3 Materials and methods

3.1 Procurement of 'Bartlett' pears

'Mountain Bartlett' pears (size 135) were hand-harvested on August 5th, 2020, from an orchard in Mendocino County, Northern California after they had matured to a firmness of 80.1-89.0 N (18-20 lbs_f). Pears were placed in plastic bins and transferred to Scully Packing (Exeter, CA, USA) on the same day of harvest. At the packing house, the pears were washed without antimycotic treatment in a float tank with 100-150 ppm chlorinated water maintained at a pH of 6-7. The pears were transferred to a sorting platform and then hand-packed into 16.3 kg tight-fill cartons measuring 0.43 m (length) x 0.33 m (width) x 0.22 m (height). The cartons of pears were then forced-air cooled to -1 ºC. Thirty-seven boxes of 'Bartlett' pears were then transported to Chapman University (Orange, CA, USA) by refrigerated truck at 0 ºC.

3.2 Experimental design

The experimental design and procedure are illustrated in **Figure 8**.

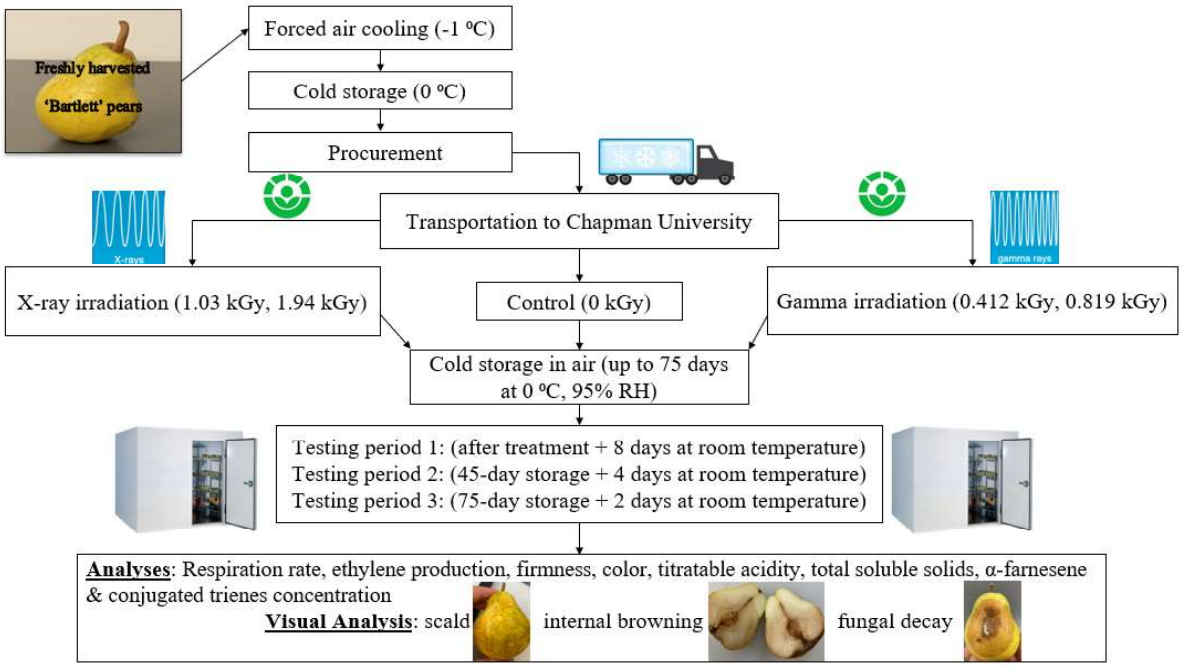

Figure 8. Procurement, irradiation, storage, and analyses of 'Bartlett' pears

3.3 'Bartlett' pear irradiation treatment

Twelve boxes of pears were transported to Sterigenics (Corona, CA, USA) for gamma irradiation. Six boxes were irradiated at target doses of 0.4 kGy and six boxes at 0.8 kGy. For the target dose of 0.4 kGy, the average absorbed dose 0.412 kGy was calculated from 18 dosimeters at an irradiation temperature of 23 °C. The minimum dose was 0.35 kGy, and the maximum dose of 0.45 kGy providing a dose uniformity ratio (DUR) of 1.29. For the target dose of 0.8 kGy, the average absorbed dose of 0.812 kGy was calculated from 18 dosimeters at an irradiation temperature of 23 °C. The minimum dose was 0.68 kGy, and the maximum dose was 0.92 kGy providing a DUR of 1.35. The dose rate was 0.23-0.26 kGy/h.

Twelve boxes of pears were transported to Steri-Tek (Fremont, CA, USA) for x-ray irradiation, which was accomplished by passing the fruit in a single pass using two MEVEX (Stittsville, Canada) e-beam accelerators with tantalum x-ray convertors (7.5 MeV, 30 kW)

facing one another. Initial dose mapping was conducted on two cases using seven B3WINDOSE dosimeters (GEX corporation) placed at various locations on the surface of pears within the box. During treatment, the racks were conveyed at different speeds in order to achieve the target doses of 0.4 kGy and 0.8 kGy. However, the pears received more than twice the target dose during treatment due to an incorrect conveyor speed calculation. For the target dose of 0.4 kGy, the average absorbed dose was 1.03 kGy calculated from seven dosimeters at an irradiation temperature of 25 °C. The minimum dose was 1.0.3 kGy, and the maximum dose was 1.04 kGy providing a DUR of 1.01. For the target dose of 0.8 kGy, the average absorbed dose was 1.94 kGy calculated from seven dosimeters at an irradiation temperature of 25 °C. The minimum dose was 1.88 kGy, and the maximum dose was 1.99 kGy providing a DUR of 1.06. The dose rate was approximately 50 kGy/h. Boxes containing control pears were not irradiated and remained in the refrigerated truck. After irradiation treatment, the irradiated and untreated pears were transported to Chapman University for storage.

3.4 Storage of irradiated and nonirradiated ‘Bartlett’ pears

All boxes of irradiated and nonirradiated ‘Bartlett’ pears were placed in cold storage (-1-0 ºC, 95% relative humidity) for up to 75 days. There were three testing periods in this study. On days 1, 45, and 75, two boxes containing 200 randomly selected fruit from each treatment were removed from cold storage and placed at room temperature. Ethylene production and respiration rates were measured each day as the pears ripened. When the pears reached their climacteric peak (based on ethylene production and respiration rate), the pears were evaluated for physical and chemical parameters as described section 3.6. Thus, in testing period one, pears stored for one day in cold storage ripened in eight days at room temperature. During testing period two,

pears stored for 45 days in cold storage ripened within four days at room temperature. In testing period three, pears stored for 75 days ripened in two days at room temperature.

3.5 Ethylene production and respiration rate

The ethylene production and respiration rates were measured every day after the fruit was removed from cold storage and until after their climacteric peak had passed. With modifications from Sea et al. (2015), nine pears from each treatment were selected from their corresponding boxes using simple random sampling. The weight and volume of each set of nine pears were recorded. The pears were warmed to room temperature and placed into mason jars (three jars per treatment containing nine pears each) that were sealed with rubber stoppers. Each jar was wrapped with parafilm to prevent gas leakage once they were sealed with a stopper. Ethylene and carbon dioxide were allowed to accumulate in the jar headspace for one hour.

A 10 mL sample was extracted from the headspace using a 10 mL syringe and injected into a gas chromatograph (SRI Instruments, Torrance, CA, USA) that was equipped with a thermal conductivity detector (TCD) and flame ionizing detector (FID). The TCD detected the bonds between carbon and oxygen in carbon dioxide. Ethylene was detected by the FID when the flame burned the ethylene gas producing a temperature increase. The detector recorded the change in temperature as the ethylene peak on the gas chromatograph. The column temperature was 80 ºC with an internal pressure of 10 psi. The carrier gas was ultra-high purity grade compressed hydrogen gas. The gas standard was ethylene (10 ppm) and carbon dioxide (10.0 %) in nitrogen from WestAir (Anaheim, CA, USA). Three measurements per jar were taken to comprise 18 measurements per sample. Ethylene production and respiration rate were measured every day during each time period until two days after the climacteric peak. The respiration rate

and ethylene production were calculated using **Equation 1** and **Equation 2**, respectively (M. Saltveit, 2012).

Equation 1. Respiration rate

$$\frac{\text{mL CO2}}{\text{kg h}} = \left(\frac{\%\text{CO2}}{100}\right) \text{x} \frac{\text{mL}}{\text{L}} \text{ x } \frac{\text{The volume of the jar (L)}}{\text{Fruit weight (kg)}} \text{ x } \frac{1}{\text{t(h)}}$$

Equation 2. Ethylene production rate

$$\frac{\mu\text{L C2H4}}{\text{kg h}} = \left(\frac{\%\text{C2H4}}{100}\right) \text{x} \frac{\mu\text{L}}{\text{L}} \text{ x } \frac{\text{The volume of the headspace (L)}}{\text{Fruit weight (kg)}} \text{ x } \frac{1}{\text{t(h)}}$$

3.6 Quality tests of 'Bartlett' pears

3.6.1 Color

The color of pears was measured with a CM-2500d Spectrophotometer (Konica Minolta, Ramsey, NJ, USA). External measurements using the Hunter L*, a*, and b* system were taken at the midpoint between the calyx and the stem on opposite sides of 25 randomly selected samples from each treatment. L*, a*, and b* values were recorded in duplicate for each sample from each treatment (Abolhassani et al., 2013; Drake et al., 1999).

3.6.2 Texture analysis

Firmness was measured using a TAXT Plus Texture Analyzer (Stable Micro Systems, Godalming, Surrey, U.K.) equipped with a Magnus Taylor 8-mm probe. The texture analyzer was calibrated with a probe speed of 4.0 $mm \cdot s^{-1}$ and penetrated 8 mm into each sample midway between the calyx and stem with the post-test rate of 10.0 $m \cdot s^{-1}$. The peak force in Newtons (N) was recorded in duplicate for 50 randomly selected pears comprising 100 measurements from each treatment.

3.6.3 Titratable acidity

Five milliliters of pear juice were extracted using a Maximatic TS738 Juice Extractor (Maxi-Matic, City of Industry, CA, USA) and brought to a volume of 50 mL using deionized water. Using a mini titrator & pH meter for fruit juice (model number HI84532- 01, Hanna Instruments, Woonsocket, RI, USA), measurements were taken in triplicate per treatment and expressed in % malic acid using **Equation 3** (Nielsen, 2010).

Equation 3. Calculation of % malic acid

$$\% \text{ Malic acid} = \frac{\text{mL base titrant x molarity of NaOH x } 67.04}{\text{mL sample x } 10}$$

3.6.4 Total soluble solids

Total soluble solids from three separate pear juices were measured in triplicate using a handheld Cole-Parmer digital refractometer, 0-85% Brix, 1.3330-1.5100 RI (Cole Parmer, Vernon Hills, IL, USA) and recorded as Brix (°Bx).

3.7 α-farnesene and conjugated trienes concentration

Sample preparation to measure α-farnesene absorbance is illustrated in **Figure 9**.

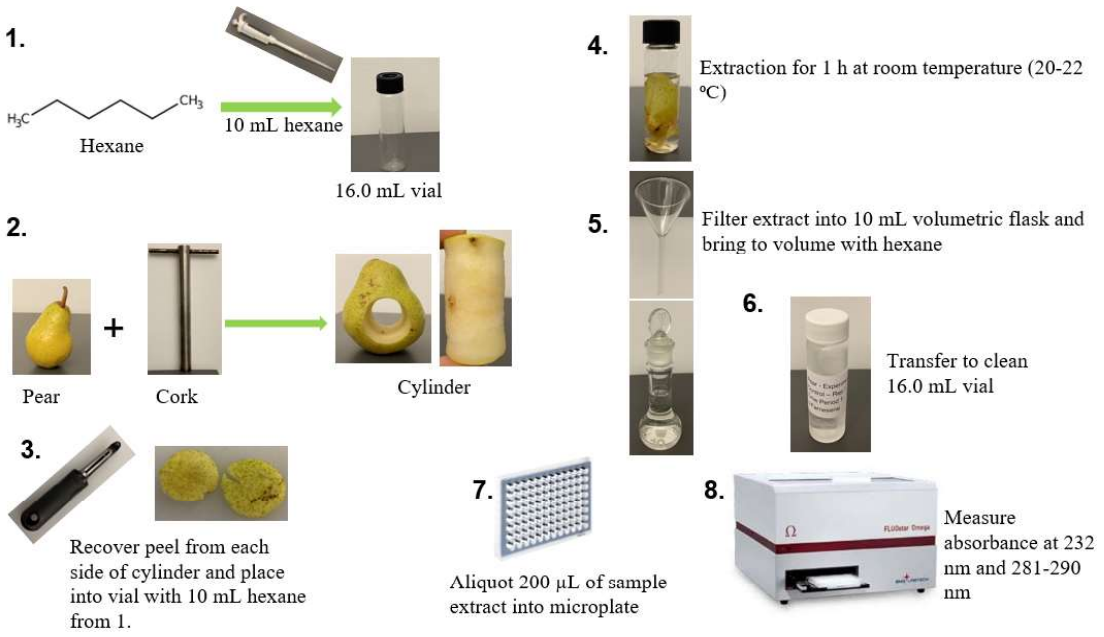

Figure 9. Sample preparation and absorbance of the α-farnesene and conjugated trienes extract.

Six pears were randomly selected from each treatment box. Ten milliliters (10 mL) of hexane were pipetted into thirty-six 16.0 mL glass vials. A cylinder of pear was cut through the equator of each pear using a cork. The peel was recovered from both sides of the cut cylinder and immersed into a glass vial with 10 mL of hexane for 1 h. The extract was filtered using quantitative ashless paper (Analytical West, Corona, CA, No. 41) into a 10 mL volumetric flask. The volume was brought to 10 mL with hexane in the volumetric flask then the extract was transferred to a clean 16.0 mL glass vial for all six pears from each treatment (Jemric, Lurie, Dumija, Pavicic, & Hribar, 2006). Two hundred microliters (200 µL) of each sample were pipetted into a 96-well quartz microplate (Hellma USA, Inc, NY, USA). The absorbance for each α-farnesene sample was read at 232 nm in a FLUOstar Omega microplate reader (BMG Labtech Inc., Cary, NC, USA) in triplicate. The absorbance for conjugated trienes was read from 281 nm – 290 nm in triplicate. The concentration of α-farnesene was calculated using the extinction coefficient $\varepsilon_{\alpha\text{-farnesene}} = 27{,}700$

while CTs were calculated using ε_{CTs} = 27,700 (Melo, 2021). Prepared samples were stored at -20 ºC until analysis.

3.8 Superficial scald severity, index, and defect analysis

Scald incidence was determined using the following scale: no injury = 0, 1-10% surface = 1, 11-32% = 2, and >33% surface = 3 (P. M. Chen, Varga, Mielke, Facteau, & Drake, 1990). The severity index was calculated as [(% fruit grade 1) + (2 x % fruit grade 2) + (3 x % fruit grade 3)]/4 (Lurie, Klein, & Arie, 1990). Senescent scald and fungal incidence were visually analyzed and recorded as absent, 1, or present, 2 (Argenta et al., 2016). The pears were cut lengthwise, and the incidence of internal browning was recorded like senescent scald and fungal incidence. Photographs were taken using a Sony ILCE-QXI lens-type compact camera (New York, NY, USA) without any additional light diffusion by placing each pear in a Neewar 24 x 24-inch Photo Studio Light Cube (purchased from Amazon, Seattle, WA, USA).

3.9 Statistical analysis

A complete 2 x 2 x 3 factorial design with two irradiation treatments at two different doses and three different times was used to test the main effects of irradiation on ethylene production, respiration rate, firmness, color, titratable acidity, total soluble solids, α-farnesene concentration, and postharvest defects of 'Bartlett' pears. Statistical analysis of the collected data was carried out using RStudio version 4.0.2 (RStudio, PBC, Boston, MA, USA) with a significance level of $\alpha = 0.05$. Any significant differences between treatments on the processing day of each testing period were determined using a two-way Analysis of Variance (ANOVA). Means from each analysis were separated using Tukey's HSD test.

4 Results

4.1 Effect of irradiation on ripening

As pears ripened at room temperature (22 °C), their respiration rate (**Figure 10**) and ethylene production (**Figure 11**) increased steadily, and the peaks coincided, showing a clear climacteric at all storage periods for all treatments. The pears reached peak respiration rate and ethylene production faster as time spent in storage increased. During testing period one, the pears reached their peak on day nine after one day in storage. After 45 days of storage (testing period two) and 75 days of storage (testing period three), the pears achieved the climacteric peak by day four and three at room temperature (22 °C), respectively.

4.1. Respiration rate and ethylene production

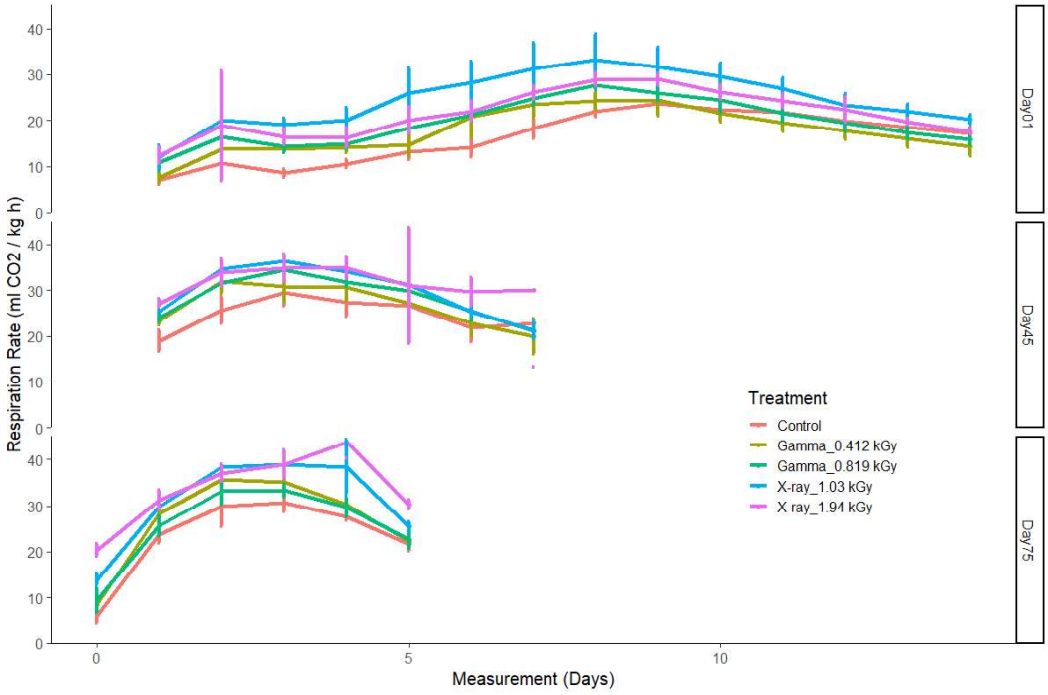

Figure 10. Respiration rate of 'Bartlett' pears treated with postharvest irradiation and stored at 1 °C for 1, 45, and 75 d.

During all three testing periods, fruit irradiated with x-ray 1.03 kGy and 1.94 kGy had the highest respiration rates, with significant differences at peak respiration ($p<0.05$). X-ray 1.94 kGy irradiated pears achieved peak respiration on the same day as the other treatments in testing period two but achieved peak respiration on day four instead of three during the testing period three.

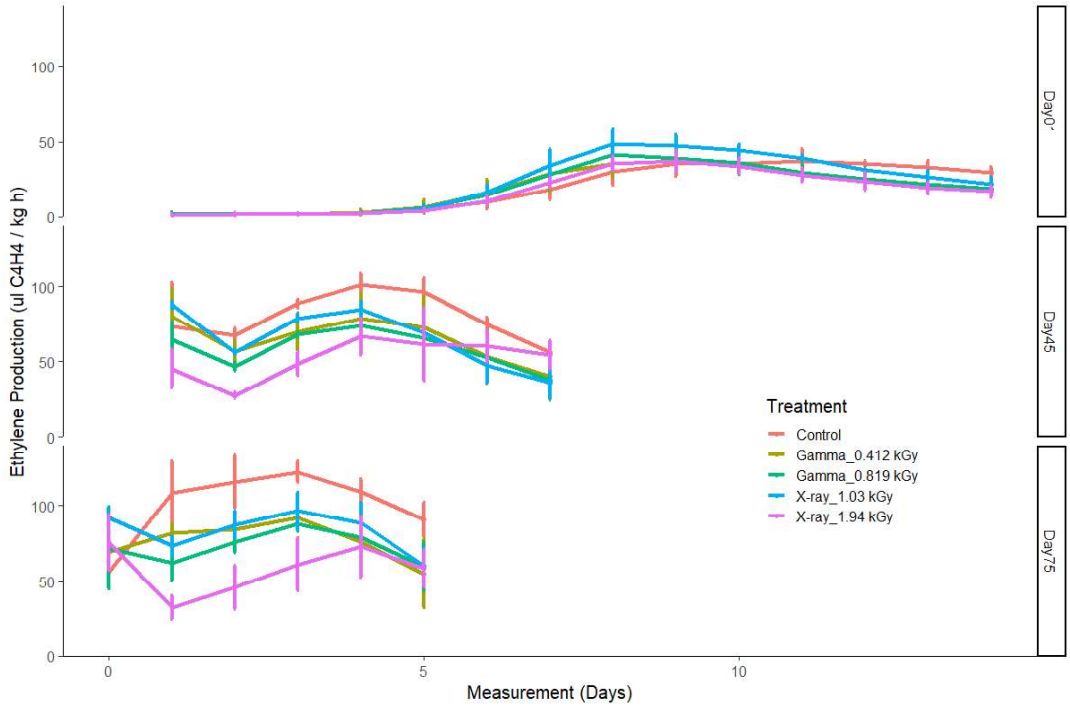

Figure 11. Ethylene production of 'Bartlett' pears treated with postharvest irradiation and stored at 1 °C for 1, 45, and 75 d.

During testing period one, non-irradiated pears had the lowest ethylene production compared to the treated pears. However, in testing periods two and three, non-irradiated pears had the highest ethylene production compared to all other treatments, and x-ray 1.94 kGy had the lowest ethylene production than the other irradiated pears, with significant differences ($p<0.05$) when the climacteric peak was reached. This set of pears also achieved peak respiration later during storage period three, indicating both lowered and delayed ripening. The gamma-treated pears and x-ray 1.03 kGy irradiated pears were clustered between the control and x-ray 1.94 kGy irradiated pears.

4.1.2 Color

Pears irradiated at the higher doses were greener and less yellow. L*, a*, and b* values of the pears measured at the climacteric peak (**Table 4**) were affected by irradiation, especially in

storage periods one and two when x-ray and gamma 0.819 kGy irradiated pears generally had lower L*, a*, and b* values than non-irradiated pears (**Figure 12**, **Figure 13**).

Table 4. Effect of irradiation and storage time on L*, a*, and b* values of 'Bartlett' pears after three storage periods: 1 day at 1 °C plus 8 days at room temperature, 45 days at 1 °C plus 5 days at room temperature, and 75 days at 1 °C plus 2 days at room temperature.

	1 d storage + 8 d			**45 d storage + 5 d**			**75 d storage + 2 d**		
Treatment	L*	a*	b*	L*	a*	b*	L*	a*	b*
0 kGy	68.36^{a}	-0.14^{bc}	51.00^{a}	75.41^{a}	6.61^{ab}	52.07^{a}	72.24^{a}	-0.13^{c}	51.39^{a}
gamma 0.412 kGy	69.07^{a}	2.56^{a}	50.87^{ab}	73.95^{abc}	7.20^{a}	51.81^{a}	72.86^{ab}	3.27^{a}	49.34^{bc}
gamma 0.819 kGy	67.49^{ab}	0.77^{bc}	49.83^{abc}	74.36^{ab}	6.02^{b}	52.17^{a}	74.17^{a}	3.02^{ab}	50.71^{ab}
x-ray 1.03 kGy	65.18^{b}	-2.07^{c}	47.72^{c}	73.32^{bc}	6.15^{b}	51.04^{a}	73.35^{a}	3.45^{a}	49.92^{ab}
x-ray 1.94 kGy	65.75^{b}	-1.60^{c}	48.82^{bc}	72.24^{c}	5.08^{c}	50.51^{a}	70.48^{b}	1.11^{bc}	47.90^{c}

$^{+}$ Treatments with the same letter in the same column are not statistically significant ($p<0.05$).

In the third storage period, the pears ripened quickly, and there was little difference in color due to treatment (**Figure 14**). Chroma and hue were not different among treatments (data not shown).

Figure 12. 'Bartlett' pears after 1-day storage at 1 °C plus 8 days at room temperature (22 °C).

Figure 13. 'Bartlett' pears after 45-day storage at 1 °C plus 4 days at room temperature (22 °C).

Figure 14. 'Bartlett' pears after 75-day storage at 1 °C plus 3 days at room temperature (22 °C).

4.1.3 Texture

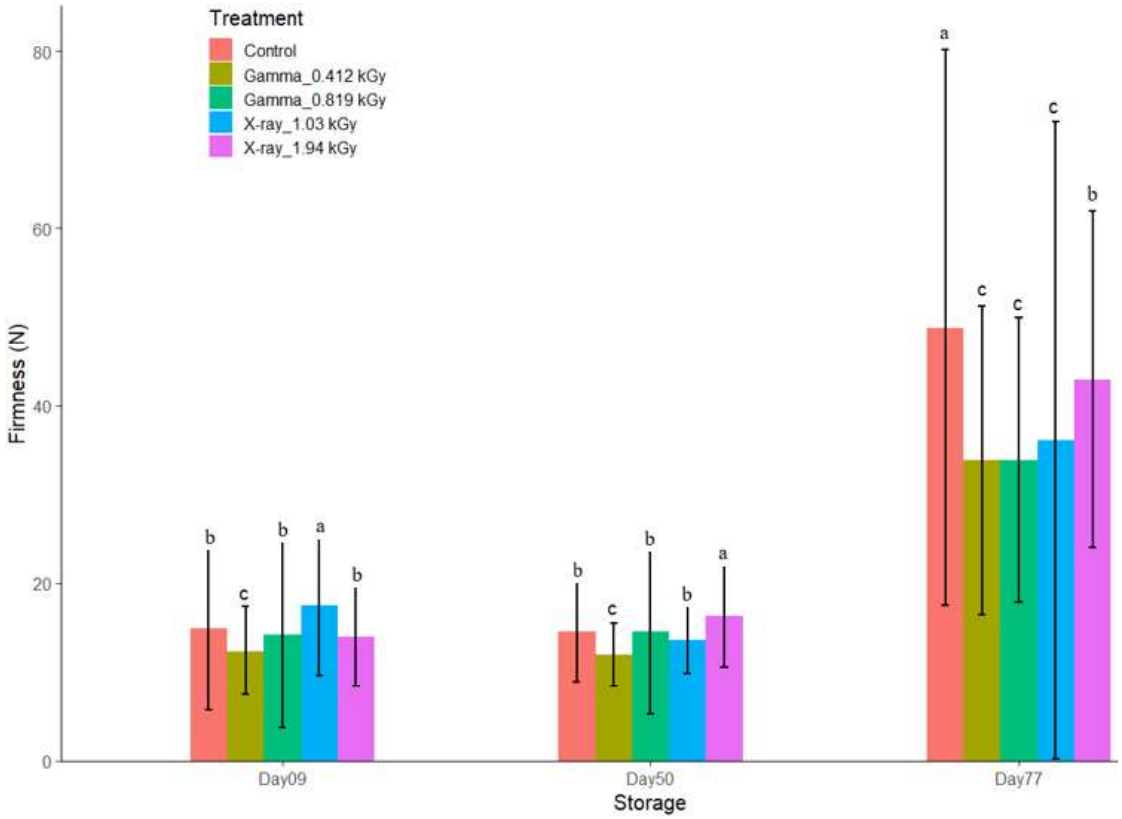

Figure 15. Effect of irradiation and storage time on firmness (N) of 'Bartlett' pears after three storage periods: 1 day at 1 °C plus 8 days at room temperature, 45 days at 1 °C plus 5 days at room temperature, and 75 days at -1 °C plus 2 days at room temperature. Results are represented as mean values and error bars show standard error of duplicate measurements on fifty pears.

During storage periods 1 and 2, there was little change in firmness of the fruit and small differences between treatments (**Figure 15**). However, the pears were approximately three times as firm as they ripened during storage period 3 than in storage periods 1 and 2. The variability in pear firmness was also much larger in storage period 3, indicating that the pears did not ripen uniformly after 75 days in storage.

4.1.4 Titratable acidity and total soluble solids content

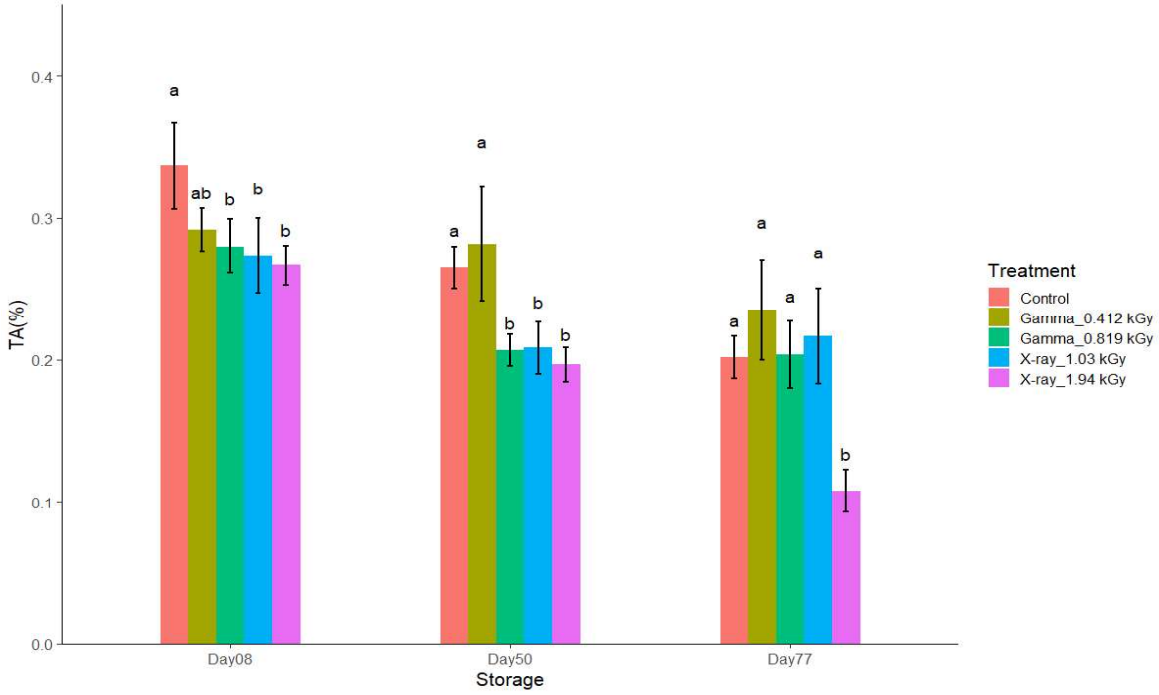

Figure 16. Effect of irradiation and storage time on titratable acidity (TA%) of 'Bartlett' pears after three storage periods: 1 day at 1 °C plus 8 days at room temperature, 45 days at 1 °C plus 5 days at room temperature, and 75 days at 1 °C plus 2 days at room temperature. Results are represented as mean values and error bars show standard deviation.

Irradiation decreased titratable acidity (TA), which was evident during all storage periods, most notably for x-ray 1.94 kGy irradiated pears, which was 21% lower than the control in the first time period and 47% by the third time period (**Figure 16**). Titratable acidity of the pears showed a decreasing trend during storage, likely due to the shorter time required for ripening.

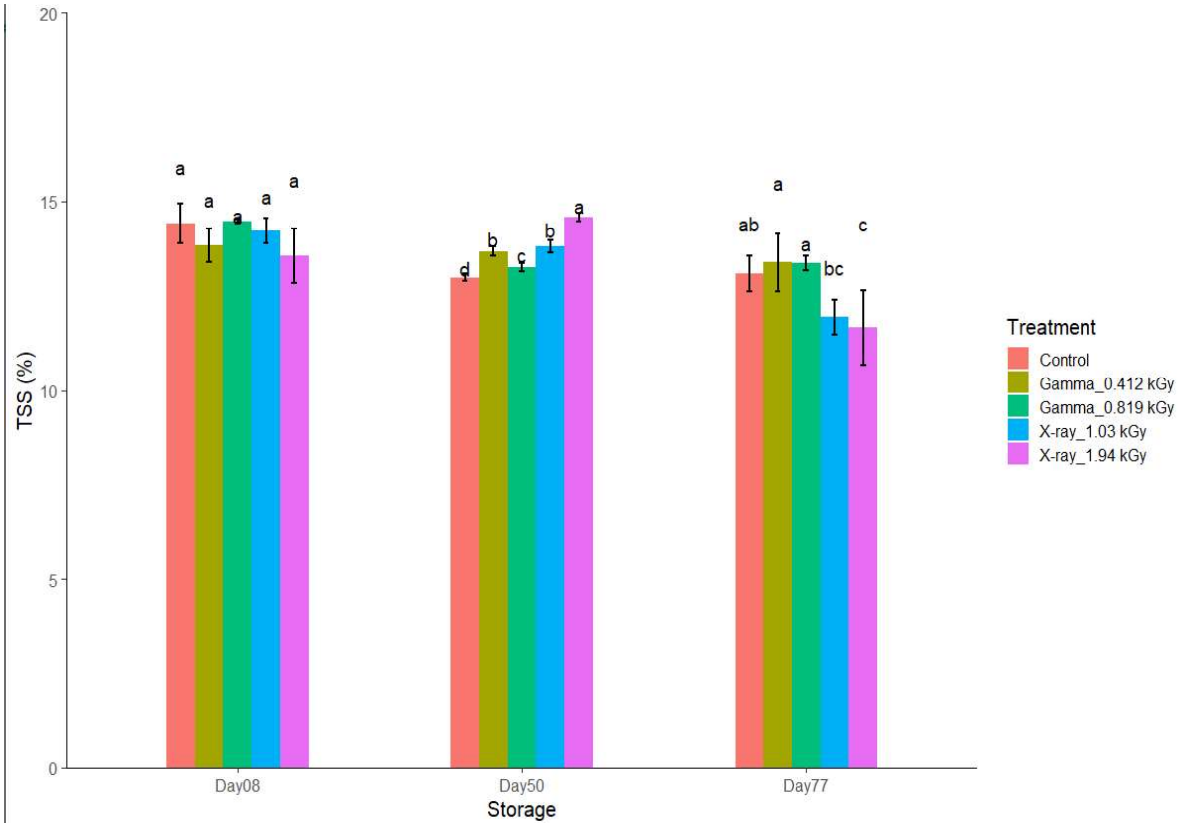

Figure 17. Effect of irradiation and storage time on total soluble solids (TSS%) of 'Bartlett' pears after three storage periods: 1 day at 1 °C plus 8 days at room temperature, 45 days at 1 °C plus 5 days at room temperature, and 75 days at 1 °C plus 2 days at room temperature. Results are represented as mean values and error bars show standard deviation.

There was a 9% decrease in TSS content of non-irradiated pears between storage periods one and two, but no change between storage periods two and three (**Figure 17**). There was little difference in the total soluble solids content among treatments except for x-ray irradiated pears in storage period three when x-ray 1.03 kGy and 1.94 kGy irradiated pears were 17% and 19% lower in TSS content, respectively than non-irradiated pears. Gamma irradiated pears showed no TSS content differences due to irradiation or storage time compared to non-irradiated pears which showed a decrease in TSS content after 45 days.

4.2 Scald incidence and severity

The x-ray irradiated pears did not exhibit any superficial scald after 75 days in storage plus two days at room temperature (**Table 5**, **Table 6**). Gamma-irradiated pears showed some superficial scald by the end of the study, but less than non-irradiated pears.

Table 5. Scald incidence of 'Bartlett' pears after three storage periods: 1 day at 1 °C plus 8 days at room temperature, 45 days at 1 °C plus 5 days at room temperature, and 75 days at 1 °C plus 2 days at room temperature at room temperature.

Treatment	Day 9	Day 50	Day 77
0 kGy	0%	0%	33%
gamma – 0.412 kGy	0%	0%	9%
gamma – 0.819 kGy	0%	0%	0.7%
x-ray – 1.03 kGy	0%	0%	0%
x-ray – 1.94 kGy	0%	0%	0%

Table 6. Scald index of 'Bartlett' pears after three storage periods: 1 day at 1 °C plus 8 days at room temperature, 45 days at 1 °C plus 5 days at room temperature, and 75 days at 1 °C plus 2 days at room temperature at room temperature.

Treatment	Day 9	Day 50	Day 77
0 kGy	0	0	0.17
gamma – 0.412 kGy	0	0	0.02
gamma – 0.819 kGy	0	0	0.002
x-ray – 1.03 kGy	0	0	0
x-ray – 1.94 kGy	0	0	0

4.2.1 α-farnesene and conjugated trienes concentration

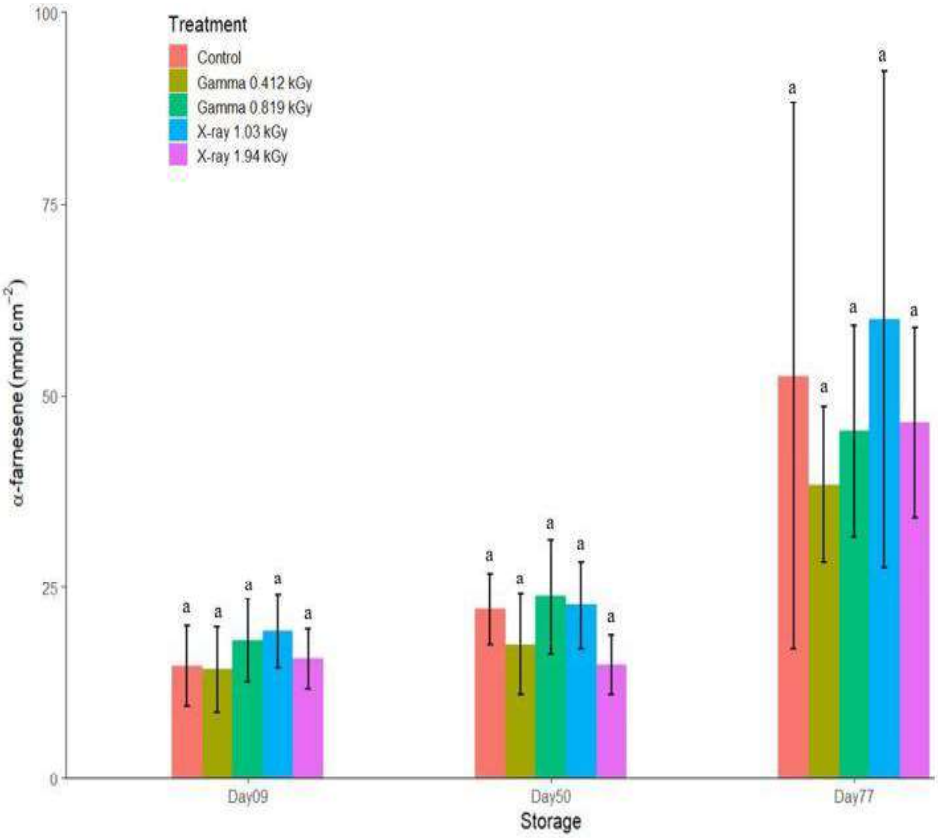

Figure 18. Effect of irradiation and storage time on alpha-farnesene concentration of 'Bartlett' pears after three storage periods: 1 day at 1 °C plus 8 days at room temperature, 45 days at 1 °C plus 5 days at room temperature, and 75 days at 1 °C plus 2 days at room temperature. Results are represented as mean values and error bars show standard deviation.

There were no significant differences ($p>0.05$) among treatments during any of the testing periods (**Figure 18**). There was an increase in alpha-farnesene concentration in all irradiated pears between testing periods two and three. The high standard deviations on day 77 are reflective of the high variability in alpha-farnesene concentration in the pear peels.

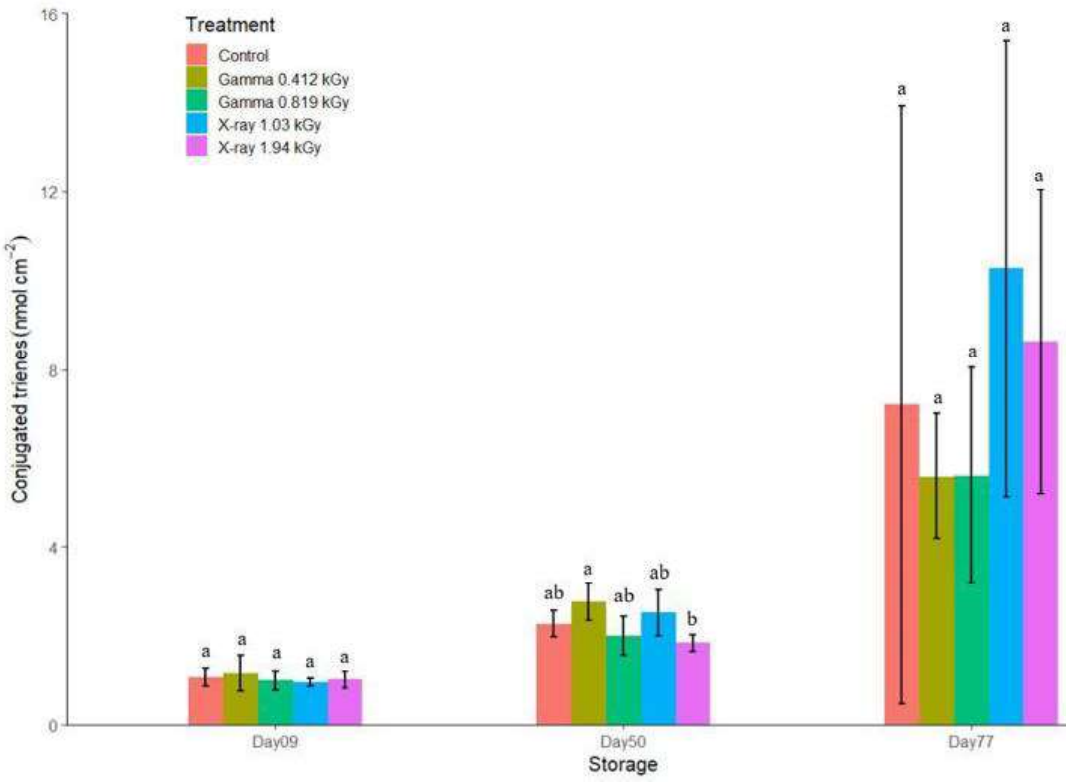

Figure 19. Effect of irradiation and storage time on conjugated triene content of 'Bartlett' pears after three storage periods: 1 day at 1 °C plus 8 days at room temperature, 45 days at 1 °C plus 5 days at room temperature, and 75 days at 1 °C plus 2 days at room temperature. Results are represented as mean values and error bars show standard deviation.

There was an increase in conjugated trienes content in all fruit as storage time increased (**Figure 19**). Similar to the alpha-farnesene content results, the conjugated trienes content had a large increase between storage periods two and three. Similar to the alpha-farnesene concentrations on day 77, the high standard deviations on day 77 were reflective of the high variability in conjugated trienes in the peels.

4.3 Other defects – fungal rot, internal browning, senescent scald

The pears had fungal rot in the second and third storage periods, and the incidence was dependent on dose. The control had the highest level of rot of 18%, followed by the gamma-treated

pears. X-ray irradiated pears exhibited 1-3% rot after 45 days in storage plus five days at room temperature (**Table 7**). In storage period three, 5% of the 0.412 kGy gamma-irradiated pears showed signs of internal browning. Neither the control nor any of the other irradiated pears showed signs of internal browning. Also, in storage period three, 6% of the non-irradiated pears developed senescent scald, but none of the irradiated pears exhibited this defect.

Table 7. Percentage of 'Bartlett' pears with fungal rot after three storage periods: 1 d at 1 °C plus 8 d at 22 °C (9 d), 45 d at 1 °C plus 5 d at 22 °C (50 d), and 75 d at 1 °C plus 2 d at 22 °C (77 d).

Treatment	9 d	50 d	77 d
0 kGy	0	18	35
Gamma 0.412 kGy	0	9	2
Gamma 0.819 kGy	0	12	13
X-ray 1.03 kGy	0	3	0
X-ray 1.94 kGy	0	1	0

5 Discussion

5.1 Effect of ionizing irradiation and storage on ripening

5.1.1 Respiration rate and ethylene production

Longer storage time at cold temperature led to an increase in respiration rate of the pears when they ripened at room temperature (**Figure 10**), similar to increased respiration rates observed in 'Bartlett' pears following cold storage for up to 12 weeks by previous investigators (Agar et al., 2000; Whitaker et al., 2009). Continued synthesis of ethylene during cold storage results in higher respiration and faster ripening when pears are taken out of cold storage (Agar et al., 2000).

Irradiation increased respiration rate in a dose-dependent manner. Irradiation-induced increase in respiration rate has been observed previously in 'Bartlett' pears irradiated at 0.4 kGy using x-rays (Sea et al., 2015) and 0.940 kGy using gamma (Tu, 2020). Increased respiration rates in 'Fuji' apples exposed to 0.377 kGy and 1.15 kGy gamma irradiation were also observed by Kheshti et al. (2019). Higher respiration rates in response to irradiation treatment suggest that irradiation treatment results in a stress response similar to wounding (Gunes, Watkins, & Hotchkiss, 2000; Sea et al., 2015). Irradiation can generate reactive oxygen species (ROS) such as hydrogen peroxide and hydroxyl radicals which can interfere with cellular metabolism. The stimulation of protective defense mechanisms occurs when irradiation-generated ROS quickly reacts with the structure and functionality of organic molecules (Ahuja et al., 2014). Enzymes such as ascorbate peroxidase and glutathione reductase and antioxidants such as ascorbic acid and reduced glutathione are examples of protective defense compounds that work together to scavenge

ROS and help ensure cellular homeostasis of plant tissue (Das and Roychoudhury, 2014). However, the synthesis of these components requires the production of cellular energy through respiration. The need to scavenge ROS generated by irradiation increases the respiration to produce the cellular energy needed to synthesize the required defense components (Marcu et al., 2013).

Irradiation lowered ethylene production (**Figure 11**). Fruit treated at the highest doses had the lowest ethylene production, although all the fruit generally experienced the climacteric peak on the same day. Irradiation-induced decreases in ethylene production have been observed in late harvest 'Bartlett' pears treated with 0.400 kGy x-ray irradiation (Sea et al., 2015), 'Granny Smith' apples treated with 0.310 kGy gamma irradiation (Melo et al., 2021), and 'Bartlett' pears treated with 0.470 kGy and 0.940 kGy gamma irradiation (Tu, 2020).

Ethylene synthesis begins with the amino acid methionine which is converted to S-adenosyl-L-methionine (SAM) by the addition of adenine. Two key enzymes that regulate ethylene production are 1-aminocyclopropane-1-carboxylate synthase (ACS) and 1-aminocyclopropane-1-carboxylate oxidase (ACO). ACS catalyzes the conversion of SAM to 1-amino-cyclo-propane carboxylic acid (ACC) and is considered the rate-limiting step in ethylene production. ACO oxidizes ACC to ethylene (Saltveit, 1999; Bulens et al., 2011). There is a pronounced increase in the rate of ACO activity in ripening pears and in response to ethylene (Bulens et al., 2011). It has been found that x-ray irradiation at 0.310 kGy and 0.1 kGy decreases ACO activity in 'Granny Smith' apples after 90 and 180 days in cold storage (Melo et al., 2021), in 'Granny Smith' apples gamma-irradiated at 0.24 kGy after 90 and 150 days (Loayza et al., 2020), and in papayas irradiated with 0.5 kGy of gamma irradiation (D'Innocenzo and Lajolo, 2001) thus resulting in decreased ethylene production in the fruit.

The dose-dependent decrease in ethylene production of the fruit in our study was likely due to the inhibition of protein synthesis and subsequent enzyme activity by ROS and free radicals. Ionizing radiation can inhibit enzyme activity by modifying the secondary and tertiary structures of proteins and inhibiting protein synthesis itself (Braunstein et al., 2009; Harder et al., 2016). The structural unfolding of proteins resulting from irradiation decreases activity through the radiolytic breakdown of peptide bonds and oxidative deamination of component amino acids (Cecarini et al., 2007; Reisz et al., 2014). Irradiation can inhibit protein synthesis by acting upon the initiation step in translation and can alter gene expression at the mRNA translation level, thus changing the corresponding protein (Lü et al., 2006).

A dose-dependent decrease of ethylene delayed the ripening of the pears in our study, as evident by the slower change in color of 1.94 kGy x-ray irradiated pears (**Table 4**, **Figure 13**). Wani et al. (2008), Abolhassani et al. (2013), and Tu (2020) also observed delay in ripening of pears treated with irradiation in the range of 0.47-1.75 kGy.

5.1.2 Color and appearance

Ethylene mediates chlorophyll degradation and the appearance of yellow colors in climacteric fruit. By removing ethylene or inhibiting its action, color changes during storage can be delayed, and shelf-life can be extended (Saltveit, 1999). As pears ripen, their color changes from green to yellow, their L* values decrease, and their a* and b* values increase. This color change from green to yellow results from a multi-step enzymatic process of chlorophyll breakdown by chlorophyllase and chlorophyll b reductase resulting in a pigment conversion of green chlorophyll to red and yellow carotenoids (Cheng et al., 2012; Charoenchongsuk et al., 2018). In our study, irradiated pears at the higher doses were greener (lower a* values) and less yellow

(lower b* values) than non-irradiated pears at the climacteric peak in storage periods one and two (**Table 4**). During storage period 3, all the pears ripened within 2-3 days, and the color differences were not evident (**Figure 14**).

Abolhassani et al. (2013) and Tu (2020) also observed that irradiated 'Bartlett' pears remained greener longer than control pears. The authors attributed the delay of degreening to the inhibition of chlorophyll degradation. Free radicals and ROS can oxidize the phytyl chain of chlorophyll, resulting in the appearance of yellow colors and the formation of isophytol (Nguyen et al., 2021). Irradiation can inhibit chlorophyllase enzyme activity that converts chloroplast into chromoplasts by producing free radicals that are stress signals to inhibit chlorophyll degradation resulting in higher chlorophyll retention (Fan and Thayer, 2001; Sea et al., 2015). Free radicals can act as stress signals by triggering an antioxidant response which scavenges and removes harmful products such as free radicals before they can cause cellular damage thus resulting in inhibition of chlorophyll degradation (Fan and Thayer, 2001; Hussain et al., 2010). Wani et al. (2008) found that higher chlorophyll retention was achieved in pears treated with 1.5-2.0 kGy gamma irradiation, which they attributed in part to the inhibitory effects of irradiation on chlorophyllase activity. Nguyen et al. (2021) observed that chlorophyll degradation was delayed in green mangoes treated with electron beam irradiation by decreasing ROS activity through activating the antioxidant capacity of catalase, ascorbate peroxidase, and glutathione.

5.1.3 Texture

Ethylene production and its perception are needed to begin and to continue the softening process in pears as they ripen (Hiwasa et al., 2003). In climacteric fruit, this softening of texture correlates with increased ethylene biosynthesis until the climacteric peak is reached. After this

point, ethylene production starts to decrease, but pears continue to soften until they senesce. As pears ripen, enzymes such as protopectinase and pectin methylesterase convert the insoluble fractions of the cell walls into their soluble forms resulting in a softer texture of the fruit (Makkumrai et al., 2014; Zdunek et al., 2016).

There was little to no change in firmness in the fruit in the first storage period and minor differences between treatments after 45 days of storage (**Figure 15**). However, the pears were approximately three times as firm after 75 days of storage, with high variability in firmness values. Some pears had softened while others remained firm as the pears reached peak ripeness within two days at room temperature. A storage period of 75 days (approximately 11 weeks) is likely past the storage period for 'Bartlett' pears, and the rapid onset of ripeness resulted in accelerated senescence. Agar et al. (2000) found that 'Bartlett' pears reached the end of their storage life after 10 weeks and Tu (2020) reported that 'Bartlett' pears were beyond their storage life after 12 weeks.

Irradiation, even at a dose of 1.94 kGy, did not impact firmness of the pears. Tu (2020) also observed no difference in the firmness of pears treated with gamma irradiation up to 1 kGy. In contrast, irradiated pears (0.4-0.8 kGy) have been observed to be firmer than non-irradiated pears (Abolhassani et al., 2013; Sea et al., 2015; Tu, 2020) attributed to decreased levels of ethylene that inhibited ripening (hence softening) in irradiated pears. Despite the significantly lower ethylene levels in the irradiated pears in our study, pear firmness was not impacted, suggesting that low levels of ethylene production did not inhibit pear ripening.

5.1.4 Titratable acidity and total soluble solids

Titratable acidity decreased during storage in the control pears as organic acids were converted to sugars and their derivatives in respiration (Nath et al., 2012). Irradiation significantly reduced

($p<0.05$) the TA of pears (**Figure 16**), similar to decreased TA observed in irradiated fruit (Abolhassani et al., 2013; Kheshti et al., 2019; Tu, 2020, Loayza et al., 2020; Chang, 2021). This was attributed to the higher respiration rate of irradiated fruit that uses organic acids as substrates to form new compounds (Abolhassani et al., 2013; Sea et al., 2015; Tu, 2020). Organic acids can be converted back to carbohydrates, or their carbon skeletons can be reused to synthesize amino acids depending on the metabolic needs to maintain homeostasis during ripening (Igamberdiev and Eprintsev, 2016). The decline in acidity is primarily because of the loss of malic acid and, to a lesser degree, citric acid, which takes part in climacteric respiration (Akhavan and Wrolstad, 1980). Kheshti et al. (2019) suggested that exposure to high-dose irradiation could temporarily decrease organic acid content by inhibiting enzymes along the glycolytic pathway.

In contrast to TA, there was little change in TSS content among treatments between storage periods except for x-ray irradiated pears at 1.03 and 1.94 kGy (**Figure 17**). Similarly, no change in TSS has been reported in 'Fuji' apples (Drake et al., 1999; Drake et al., 2003) 'Granny Smith' apples (Loayza et al., 2020; Chang, 2021), 'Anjou' pears, 'Bosc' pears (Drake et al., 2003) and 'Bartlett' pears (Tu, 2020; Sea et al., 2015) irradiated at 0.25-.0.95 kGy. Yet, an observed decrease in TSS has been reported in 1.0 kGy irradiated grapes (Kang et al. 2012) and guavas (Singh and Pal, 2009) after 2-3 weeks of storage. Kaur and Dhillon (2015) reported that a gradual increase in TSS of non-irradiated pears in storage for up to 60 days could be attributed to the hydrolysis of starch and pectins into mono and disaccharides that prepare the fruit for senescence.

In our study, 1.03 and 1.94 kGy treated pears had the lowest TSS, and highest respiration rates, during storage period three. Similarly, Abolhassani et al. (2013) observed that irradiation at a higher dose of 0.8 kGy significantly decreased TSS in gamma-irradiated 'Bartlett' pears as compared to 0.4 and 0.6 kGy pears. Sea et al. (2015) also observed a decrease in the TSS content

of late-harvest pears treated with 0.4 kGy x-ray irradiation, but not in early-harvest pears, which they attributed to the higher respiration rates in the late-harvest irradiated pears. The lowered TSS content and greater reduction of TA observed in 1.94 kGy x-ray irradiated pears correlates with a significantly higher respiration rate of these pears when they reached their climacteric in our study. These results suggest that at low irradiation doses, organic acids may be the substrates of preference rather than sugars for respiration and that the fruit draws on sugars at higher doses (Akhavan and Wrolstad, 1980; Tu, 2020).

5.2 Effect of storage and irradiation on fungal rot, internal browning, and senescent scald

Fungal rot was suppressed (**Table 7**) at the higher doses (1.03 and 1.94 kGy) but not at the lower doses (0.412 and 0.819 kGy). Tu (2020) also reported that fungal rot was suppressed in 92% of pears irradiated with 940 Gy, whereas fungal rot was suppressed in only 72% of the fruit treated with 470 Gy after 90 days of cold storage. Geweely and Nawar (2006) observed the percentage of spore germination of *B. cinerea* and *P. expansum*, significant postharvest pathogens of pears (Mitcham, 1996), decreased with increases in gamma irradiation doses from 0.75-1 kGy. Geweely and Nawar (2006) suggested that because the fungi had thin cell walls, gamma irradiation could impact cellular DNA with increasing dose as ROS, peroxides, and free radicals from the radiolysis of water can cause breaks within the DNA strands. Jeong et al. (2017) observed that *P. expansum* infection decreased by 60% in 'Niitaka' pears with an increase in dose from 100 to 200 Gy, which they suggested was the result of enhanced enzyme activity of enzymes related to disease resistance such as β-1, 3-glucanase, phenylalanine ammonia lyase (PAL), peroxidase, and polyphenol oxidase, thereby inhibiting fungal infection.

Internal browning was only observed in pears irradiated with 0.412 kGy after 75 days of storage. Internal browning in fruit irradiated with similar doses have previously been observed in 'Gala' apples (Fan and Matthias, 2001) irradiated with 0.44-1.32 kGy and pineapples (Jenjob et al., 2017) irradiated with 0.4-0.6 kGy. In contrast to our results, Melo et al. (2021) found that internal browning was evident in 'Granny Smith' apples treated with 1 kGy after 90 days of storage whereas it was not evident in control and 0.31 kGy treated fruit. However, after 180 days, internal browning increased in all treatments (control, 0.31 kGy, and 1 kGy). During storage, localized hypoxic conditions in the fruit can lead to oxidative stress and metabolic shifts towards energetically less efficient fermentation pathways. This shift leaves the fruit unable to repair damage caused by reactive oxygen species to the cellular membrane. As this damage to the membrane occurs, enzymatic oxidation of phenolic substrates such as chlorogenic acid and catechin occurs, leading to browning (Pedreschi et al., 2009). The development of internal browning in irradiated fruit is suggested to be the result of the accumulation of phenolic compounds produced by an increase in PAL activity resulting in internal browning (Reyes and Cisneros-Zevallos, 2007; Jenjob et al., 2017).

Senescent scald only developed in 6% of control pears after 75 days of storage. Tu (2020) reported that 5% of control pears developed senescent scald after 90 days, whereas 470 and 940 kGy irradiated pears had an occurrence of 0% and 3%, respectively. Lum et al. (2017) observed that ethylene production might be associated with senescent scald as 1-MCP, the ethylene inhibitor, was correlated with a decrease in senescence-related defects in stored pears (167 d). This observation suggests that as increased doses in irradiation lowered ethylene production in treated pears, the development of senescent scald was reduced.

5.3 Effect of storage on superficial scald

Superficial scald was observed in control pears only after 75 days in cold storage. While 33% of the control pears exhibited superficial scald (**Table 5**), the scald severity was low (0.17,**Table 6**). The extent of superficial scald in pears varies widely. For example, Drake et al. (1999) observed an increase in scald as storage time increased with 7.3% of non-irradiated 'Anjou' pears exhibiting scald after 60 days of storage and 19% by 120 days of storage. Whitaker et al. (2009) observed high levels of superficial scald in California 'Bartlett' pears ranging from 50% of superficial scald in an early harvest and 80% in a later harvest after 98 days of storage with corresponding severity of 1.2 and 1.3, respectively. In contrast, Tu (2020) observed that only 15% of the control pears exhibited scald after 90 days of storage in 'Bartlett' pears similar to the present study, which had a severity index of 0.17.

5.4 Scald as influenced by alpha farnesene (AF) and conjugated trienes (CT) content

Superficial scald can develop on 'Bartlett' pears because of a high accumulation of conjugated trienes, the oxidation products of alpha-farnesene (**Figure 20**). The accumulation of CT can result in cell damage and death in the peel of the fruit (Whitaker et al., 2009). As the α-farnesene content begins to accumulate, so too does the CT content, which can induce the necrosis and cell damage of hypodermal cortical tissue responsible for superficial scald (Lurie & Watkins, 2012). In this study, AF concentration remained constant in the first two storage periods and more than doubled in the third storage period (after ten weeks of storage), which is also when scald was evident (**Figure 18**). CT concentration doubled between storage periods 1 and 2 and increased by a factor of three during storage period three (**Figure 19**). Low-temperature storage of pears susceptible to superficial scald synthesizes AF at a high rate during the initial two to three months

of cold storage. After this time in cold storage, the AF concentration declines, and the CT concentration increases to a maximum concentration within 4-6 months in storage (Gapper, Bai, & Whitaker, 2006).

Whitaker et al. (2009) reported that AF concentrations in the peel of California and Washington grown 'Bartlett' pears sharply increased between 4-8 weeks of storage at -1 °C and that CT content of the fruit peaked at 12 weeks. In their study, California-grown 'Bartlett' pears exhibited higher AF and CT levels than Washington fruit and higher scald incidence. However, the authors pointed out that scald incidence, even in the CA grown fruit was lower than other scald-prone varieties and explained that AF was not always correlated with CT formation. They also suggested that the effect of CT was more pronounced in oxidatively compromised fruit. Gapper et al. (2006) also observed a significant increase in AF content in the peel of 'Anjou' pears between 8-12 weeks of cold storage that was correlated with 7% of control fruit exhibiting scald after 9 weeks and 100% of control fruit exhibiting slight to severe scald after 13 weeks.

Figure 20. Non-irradiated 'Bartlett' pears with superficial scald after 75 days storage (1 °C) plus 2 days room temperature (22 °C).

As AF content increases during storage, it can get oxidized into CTs as a response to oxidative stress, which triggers an antioxidant defense mechanism carried out by the increased

accumulation of chlorogenic acid. Excess chlorogenic acid can then be oxidized by PPO to maintain systemic feedback regulation of the antioxidant system of polyphenolic compounds, thus generating brown discoloration. As such, AF and CTols can be considered as triggering signals for scald symptoms rather than their direct causative agents as the oxidation of chlorogenic acid by PPO can be the major contributor to scald development (Busatto et al., 2014). Calvo et al. (2015) suggested that there is a point where a CT threshold is surpassed where scald develops independently of CT levels and may be more associated with other determinant compounds such as antioxidants rather than the overall antioxidant potential of the fruit.

5.5 Effect of irradiation on superficial scald

Irradiation lowered the incidence (**Table 5**) and severity (**Table 6**) of superficial scald of treated pears in our study. Irradiated pears did not exhibit any incidence of scald until the final testing period of the study, with 9.7% of the irradiated pears at lower doses of 0.412 kGy and 0.819 kGy exhibiting scald as compared to 33% of the control pears. Pears irradiated with 1.03 kGy, and 1.94 kGy did not exhibit scald. Inhibition of scald with increasing doses with irradiation has been previously reported. Massey et al. (1964) observed that the scald incidence of several harvests of 'Rome Beauty,' 'McIntosh,' and 'Cortland' apples decreased (to zero in some instances) as irradiation doses increased up to 1.0 kGy. Drake et al. (1999) observed that 'Bosc' pears did not exhibit scald after exposure of up to 0.90 kGy irradiation, although they saw the opposite with 'Anjou' pears where scald incidence increased with doses of 0.60-0.90 kGy. Melo et al. (2021) also observed lowered scald incidence with increased irradiation doses. After six months of storage at 0 °C, 18% of 'Granny Smith' apples treated with 0.310 kGy of irradiation developed scald while apples treated with 1.0 kGy did not (Melo et al., 2021). In contrast, Tu (2020) reported that 22%

and 24% of ‘Bartlett’ pears irradiated with 0.47 kGy and 0.94 kGy, respectively, developed scald compared to 15% in non-irradiated pears.

In irradiated pears, AF and CT content increased during storage period 3 as it did for the control pears. No scald was observed on the x-ray treated pears, and incidence of scald was low on the gamma-treated pears; thus, the decrease in the incidence of scald in irradiated pears seems to be unrelated to AF and CT content. There appears to be a correlation between ethylene production and scald formation, however. The control pears had the highest ethylene production and higher scald development, and irradiated pears had lower ethylene production and scald formation in a dose-dependent manner.

Melo et al. (2021) observed a high correlation between ethylene production, AF and CT concentrations, and scald formation in irradiated ‘Granny Smith’ apples. Irradiation at 0.310 kGy and 1.0 kGy lowered ethylene production, AF, and CT content, as well as scald formation after 90 and 180 days of storage as compared to control fruit. Tu (2020) also observed a decrease in ethylene production and AF and CT concentrations in ‘Bartlett’ pears irradiated at 0.470 and 0.940 Gy after 90 days of storage as compared to the control. However, scald formation was not reduced in the irradiated pears.

Our results suggest that AF and CT concentrations are not always a good indicator of scald formation, and ethylene production is not always a good indicator of AF and CT content. The relationship and interaction between ethylene, AF, and CT content are less evident in pears than in apples (Calvo et al., 2015). For example, increases in AF content have occurred when ‘Anjou’ pears were not producing ethylene at measurable amounts after removal from cold storage, which suggests that AF synthesis does not rely exclusively on ethylene production but rather on other

variables such as cold storage (Calvo et al., 2015). Furthermore, the oxidation products of AF are suggested to be more signaling compounds rather than causal agents of scald as cold storage of pears can also induce reactions between PPO and chlorogenic acid, leading to quinone and melanin production that can cause peel discoloration when the respective organelles storing these compounds are compromised by cold storage (Busatto et al., 2014; Giné-Bordonaba et al., 2020). During extended low-temperature storage, pears can become more and more compromised by oxidative stress, which generates ROS such as free radicals and superoxide radicals that can contribute to AF oxidation into CTs (Rao et al., 1998). The production of superoxide radicals resulting from the disruption of the mitochondrial electron flow under low-temperature conditions can accumulate and then disrupt cellular activity and metabolism (Rupasinghe et al., 2000). Thus, AF can be considered a low-temperature metabolite induced by chilling stress instead of a strong indicator of superficial scald (Lindo-García et al., 2021). Thus, scald susceptibility or resistance in 'Bartlett' pears may depend on ROS generation because of chilling and their scavenging capacity during storage through the natural antioxidant systems of the fruit (Rupasinghe et al., 2000).

The enzyme α-farnesene synthase is critical for the biosynthesis of α-farnesene. Pechous and Whitaker (2004) found that expression of *AFS1*, the gene responsible for encoding α-farnesene synthase in apples, is promoted by ethylene. *AFS1* was also found to be strongly associated with the buildup of α-farnesene in the peel of apples and other climacteric fruit such as pears (Pechous, Watkins, and Whitaker, 2005). An increase in ethylene production has been correlated with accumulated AF and CT concentration in the peel of 'Delicious' apples (Ju and Bramlage, 2000), 'Granny Smith' apples (Moggia et al., 2010), and 'Anjou' pears (Xie et al., 2014; Wang, 2016). Therefore, inhibiting ethylene synthesis should inhibit the gene expression of *AFS1* and consequently the production of AF. Yet, in this study, irradiation-induced inhibition of ethylene

production in the pears did not lower AF content. While there was an apparent reduction in ethylene production and scald formation, the impact on AF and CT was far less evident.

5.6 Differences between the effect of dose and dose rate on superficial scald

In our study, there was an inverse effect of dose on superficial scald formation. Pears treated with lower dose of gamma irradiation (0.412 kGy) had higher scald levels than pears treated at 1.94 kGy with x-rays. Pears treated at 0.819 kGy gamma irradiation and 1.0 kGy x-ray treatment had similar scald levels. Ethylene production was also similar for the gamma 0.819 kGy and x-ray 1.03 kGy irradiated pears, indicating that dose was the primary factor in lowering ethylene production and decreasing scald. Because ionizing irradiation causes oxidative stress and cellular damage, antioxidants can play a protective role at higher dose rates. Free radicals generated in irradiated pears may trigger an antioxidant response that scavenges and removes harmful products such as ROS before they can cause cellular damage. The antioxidant response may be dose-dependent, with a greater scavenging capacity at higher doses than lower doses. Yet, the relationship between antioxidant concentrations and irradiation-induced oxidative stress is complex and remains to be fully elucidated (Yamaguchi et al., 2008).

In our study, there was a three orders of magnitude difference in dose rates between gamma and x-ray. The dose rate for the gamma treatment was 0.23-0.26 kGy/h. In contrast, the dose rate for the x-ray treatment was 50 kGy/h. At higher dose rates, free radicals may be generated at higher rates, and not decay or combine sufficiently, thus leading to more significant impacts on cell activity and cell membranes. In maize, a dose rate of gamma irradiation at 9.17 Gy/min was more effective in inducing mutations than a lower dose rate of 0.013 Gy/min at the same dose (16.5 Gy) (Mabuchi and Matsumura, 1964). Similar effects were found in oats where more somatic mutations

occurred with x-rays at a higher dose rate of 8.1 Gy/min than at a lower dose rate of 1.12 Gy/min at the same dose (50 Gy) (Nishiyama et al., 1966).

Beaulieu et al. (1999; 2002) observed a 4-day extension in the shelf-life of mushrooms (*Agaricus bisporus*) treated with gamma irradiation at a dose of 2 kGy with a lower dose rate (4.5 kGy/h) and a 2-day extension with a higher dose rate (32 kGy/h). Low dose rate irradiation also resulted in greater phenolic accumulation and was more effective at delaying browning, whereas the higher dose rate resulted in a pronounced reduction in PPO activity and thickening of the cell membrane. The authors suggested that at the higher dose rate (32 kGy/h), free radicals may be generated at higher rates and not decay or combine sufficiently, thus leading to greater impacts on enzyme activity and cell membrane alteration.

On the other hand, it has been suggested that lower dose rates which require longer treatment times to achieve the target doses can allow for more ROS to be generated which can damage more molecules (Reisz et al., 2014). A low dose rate (1.00 kGy/h) for a longer time (1 h) for a target dose of 1 kGy using gamma irradiation can oxidize more ascorbic acid and carotenoids in sweet potatoes than at a higher dose rate (15.30 kGy/h) for a shorter time (Lu et al., 1989). Thomas et al. (1981) found that the thiamine content of frozen ground pork was affected by the dose and the difference in gamma and e-beam irradiation. Both modalities decreased thiamine in pork as dose increased from 15 kGy to 75 kGy. At 75 kGy, thiamine retention was 27% and 52% by gamma and e-beam irradiation, respectively. The authors suggested that with e-beam treatment, the free radicals that were produced rapidly decayed or combined after each pulse. In contrast, with gamma irradiation, the free radicals had continued to accumulate until a steady-state condition was achieved, leading to a high concentration of free radicals that resulted in greater destruction of thiamine content in frozen ground pork after gamma irradiation than with e-beam.

The lack of differences in physico-chemical parameters, scald formation, and fungal decay in the irradiated fruit treated with x-ray and gamma irradiation at similar doses indicates that differences in dose rates did not impact the fruit; rather, it was the dose. At a higher dose, greater ROS generation could have induced natural antioxidant systems of the fruit during storage and reduced scald formation.

6. Conclusion

This research shows that the higher doses of irradiation were more effective than the lower doses at lowering ethylene production and decreasing superficial scald development with little impact on fruit quality of 'Bartlett' pears. Differences in dose rates between gamma and x-ray treatment did not appear to affect fruit response. However, differences in absorbed dose made the comparison of gamma and x-ray difficult. Irradiation treatment of 1.03 kGy and 1.94 kGy successfully inhibited superficial scald in 'Bartlett' pears, while treatment at the lower doses (0.412 kGy and 0.819 kGy) resulted in scald, but the development was less than non-irradiated fruit. At higher doses, irradiation may have induced a greater antioxidant response in the fruit and their scavenging role may have decreased the negative impacts of chilling stress during the 75-day cold storage. AF and CT content was highest after 75 days of storage which is also when superficial scald was evident in the fruit. However, the inhibition of superficial scald in irradiated fruit was not correlated with AF and CT concentrations in the fruit during storage. Fungal decay was suppressed at the higher dose levels, and ripening was delayed. Future research directions include exploring the differences due to treatment modality at the same dose and identifying the optimum dose range that maximizes the positive effects of irradiation while minimizing or eliminating the detrimental impacts to the fruit in a way that might occur under commercial treatment.

References

Abolhassani, Y., Caporaso, F., Rakovski, C., & Prakash, A. (2013). The Effect of Gamma Irradiation as a Phytosanitary Treatment on Physicochemical and Sensory Properties of Bartlett Pears. *Journal of Food Science, 78*(9), S1437-S1444. https://doi.org/10.1111/1750-3841.12192.

Agar, I. T., & Mitcham, E. J. (2000). Commercial handling influences quality and ripening of Bartlett pears. *California agriculture (Berkeley, Calif.), 54*(3), 34-37. https://doi.org/10.3733/ca.v054n03p34

Ahuja, S., Kumar, M., Kumar, P., Gupta, V.K., Singhal, R.K., Yaday, A., Singh, B. (2014). Metabolic and biochemical changes caused by gamma irradiation in plants. *Journal of Radioanalytical and Nuclear Chemistry*. 300, 199-212. https://doi.org/10.1007/s10967-014-2969-5.

Al-Bachir, M. (1999). Effect of gamma irradiation on storability of apples (Malus domestica L.). *Plant Foods Hum Nutr, 54*(1), 1-11. https://doi.org/10.1023/A:1008007502187.

Argenta, L. C., Mattheis, J. P., Fan, X., & Amarante, C. V. T. (2016). Managing 'Bartlett' pear fruit ripening with 1-methylcyclopropene reapplication during cold storage. *Postharvest biology and technology, 113*, 125-130. https://doi.org/10.1016/j.postharvbio.2015.11.009.

Akhavan, I., Wrolstad, R.E. (1980). Variations of sugars and acids during ripening of pears and in the production and storage of pear concentrate. *Journal of Food Science*. 45(3), 499–501. https://doi.org/10.1111/j.1365-2621.1980.tb04085.x.

Bai, J., Yin, X., Whitaker, B. D., Deschuytter, K., & Chen, P. M. (2009). Combination of 1-Methylcyclopropene and Ethoxyquin to Control Superficial Scald of 'Anjou' Pears. *HortTechnology, 19*(3), 521-525. https://doi.org/10.21273/HORTSCI.19.3.521.

Barkai-Golan, R., & Follett, P. (2017). *Irradiation for quality improvement, microbial safety and phytosanitation of fresh produce*. London, England: Academic Press.

Barry, C. S., & Giovannoni, J. J. (2007). Ethylene and Fruit Ripening. *Journal of Plant Growth Regulation, 26*(2), 143-159. https://doi.org/10.1007/s00344-007-9002-y.

Bearth, A., & Siegrist, M. (2019). "As long as it is not irradiated" – Influencing factors of US consumers' acceptance of food irradiation. *Food quality and preference, 71*, 141-148. https://doi.org/10.1016/j.foodqual.2018.06.015.

Beaulieu, M., Béliveau, M., D'Aprano, G., Lacroix, M. (1999). Dose rate effect of γ irradiation on phenolic compounds, polyphenol oxidase, and browning of mushrooms (*Agaricus bisporus*). *Journal of Agricultural and Food Chemistry*. 47(7), 2537-2543. https://doi.org/10.1021/jf981088r.

Beaulieu, M., D'Aprano, G., Lacroix, M. (2002). Effect of dose rate of gamma irradiation on biochemical quality and browning of mushrooms Agaricus bisporus. *Radiation Physics and Chemistry*. 63(3-6):311–315. https://doi.org/10.1016/s0969-806x(01)00518-7.

Braunstein, S., Badura, M.L., Xi, Q., Formenti, S.C., Schneider, R.J. (2009). Regulation of protein synthesis by ionizing radiation. *Molecular and Cellular Biology*. 29(21), 5645-5656. https://doi.org/10.1128/mcb.00711-09.

Bruhn, C. (2017). How does Food Irradiation work? Retrieved from https://ccr.ucdavis.edu/food-irradiation/how-does-food-irradiation-work#:~:text=X%2DRay%20Facility,is%20converted%20to%20X%2Drays.&text=The%20energy%20used%20in%20food,cause%20food%20to%20become%20radioactive.

Bulens, I., Van de Poel, B., Hertog, M. L., De Proft, M. P., Geeraerd, A. H., & Nicolaï, B. M. (2011). Protocol: An updated integrated methodology for analysis of metabolites and enzyme activities of ethylene biosynthesis. *Plant Methods, 7*(1), 17-17. https://doi.org/10.1186/1746-4811-7-17.

Busatto, N., Farneti, B., Tadiello, A., Oberkofler, V., Cellini, A., Biasioli, F., . . . Costa, F. (2019). Wide transcriptional investigation unravel novel insights of the on-tree maturation and postharvest ripening of 'Abate Fetel' pear fruit. *Hortic Res, 6*(1), 32-15. https://doi.org/10.1038/s41438-018-0115-1.

Calvo, G., Candan, A.P., Civello, M., Giné-Bordonaba, J., Larrigaudière, C. (2015). An insight into the role of fruit maturity at harvest on superficial scald development in 'Beurré D'Anjou' pear. *Scientia Horticulturae*. 192(31), 173–179. https://doi.org/10.1016/j.scienta.2015.05.032.

CFAITC. (2019). Commodity Fact Sheet – Pears. *California Foundation for Agriculture in the Classroom.* Retrieved from https://cdn.agclassroom.org//ca/resources/fact/pears.pdf

Chen, P. M., Varga, D. M., Mielke, E. A., Facteau, T. J., & Drake, S. R. (1990). Control of Superficial Scald on 'Anjou' Pears by Ethoxyquin: Effect of Ethoxyquin Concentration, Time and Method of Application, and a Combined Effect with Controlled Atmosphere Storage. *Journal of food science, 55*(1), 167-170. https://doi.org/10.1111/j.1365-2621.1990.tb06044.x.

Chen, P. M. M., W.M., & Borgic, D. M. (1983). Changes in ripening behavior of 'd'Anjou' pears (P*yrus communis L.*) after cold storage. *Scientia Horticulturae*(21), 137-146.

Cheng, Y., Dong, Y., Yan, H., Ge, W., Shen, C., Guan, J., Liu, L., Zhang, Y. (2012). Effects of 1-MCP on chlorophyll degradation pathway-associated genes expression and chloroplast ultrastructure during the peel yellowing of Chinese pear fruits in storage. *Food Chemistry*. 135, 415-422. https://doi.org/10.1016/j.foodchem.2012.05.017.

Charoenchongsuk, N., Matsumoto, D., Itai, A., Murayama, H. (2018). Ripening characteristics and pigment changes in russeted pear fruit in response to ethylene and 1-MCP. *Horticulturae*. 4(3), 22. https://doi.org/10.3390/horticulturae4030022.

Crassweller, R. (2017). Fruit Harvest - Pear Maturity Indices. Retrieved from https://extension.psu.edu/fruit-harvest-pear-maturity-indices

Crisosto, C. (2020). Fruit Physiological Disorders. Retrieved from http://postharvest.ucdavis.edu/Commodity_Resources/Fruit_Physiological_Disorders/

Crisosto, C., & Mitcham, E. (2020). Asian Pear: Internal Browning. Retrieved from http://postharvest.ucdavis.edu/Commodity_Resources/Fruit_Physiological_Disorders/?uid=12&ds=822.

Das, K., Roychoudhury, A. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers in Environmental Science*. 2, 1-13. https://doi.org/10.3389/fenvs.2014.00053.

D'Innocenzo, M., & Lajolo, F. M. (2001). Effect of gamma irradiation on softening changes and enzyme activities during ripening of papaya fruit. *Journal of food biochemistry, 25*(5), 425-438. https://doi.org/10.1111/j.1745-4514.2001.tb00750.x.

De-ying, H. U. I. W. N. R.-x. S. Y.-q. L. (2011). Inhibitory Effects of 1-MCP and DPA on Superficial Scald of Dangshansuli Pear. *Agricultural Sciences in China, 10*(10), 1638-1645. https://doi.org/10.1016/s1671-2927(11)60162-4.

Deuchande, T., Larrigaudière, C., Giné-Bordonaba, J., Carvalho, S. M. P., & Vasconcelos, M. W. (2016). Biochemical Basis of CO2-Related Internal Browning Disorders in Pears (Pyrus communis L. cv. Rocha) during Long-Term Storage. *J. Agric. Food Chem, 64*(21), 4336-4345. https://doi.org/10.1021/acs.jafc.6b00740.

Dias, C., L Amaro, A., C Salvador, Â., Silvestre, A. J. D., Rocha, S. M., Isidoro, N., & Pintado, M. (2020). Strategies to Preserve Postharvest Quality of Horticultural Crops and Superficial Scald Control: From Diphenylamine Antioxidant Usage to More Recent Approaches. *Antioxidants (Basel), 9*(4), 356. https://doi.org/10.3390/antiox9040356.

Diehl, J. (1999). *Safety of Irradiated Foods*. Karlsruhe, Germany: Marcel Dekker, Inc.

Drake, S. R., Gix, R. D., & Coureau, C. (2001). Quality of 'Anjou' pears after different types of controlled atmosphere storage. *Journal of food quality, 24*(1), 27-36. https://doi.org/10.1111/j.1745-4557.2001.tb00588.x.

Drake, S. R., Sanderson, P. G., & Neven, L. G. (1999). Response of Apple and Winterpear Fruit Quality to Irradiation as a Quarantine Treatment. *Journal of food processing and preservation, 23*(3), 203-216. https://doi.org/10.1111/j.1745-4549.1999.tb00380.x.

Drake, S.R., Sanderson, P.G., Neven, L.G. (2003). Carbohydrate concentration of apples and pears as influenced by irradiation as a quarantine treatment. *Journal of Food Processing and Preservation*. 27(3), 165-172. https://doi.org/10.1111/j.1745-4549.2003.tb00509.x .

EPA. (2016). *1-Methylcyclopropene (MCP) (224459) Federal Register Notices*. Retrieved from https://archive.epa.gov/pesticides/biopesticides/web/html/frnotices_224459.html

European Food Safety, A. (2011). Review of the existing maximum residue levels (MRLs) for diphenylamine according to Article 12 of Regulation (EC) No 396/2005: Review of the existing MRLs for diphenylamine. *EFSA journal, 9*(8), 2336. https://doi.org/10.2903/j.efsa.2011.2336

Eustice, R. (2018). Acceptance, use of food irradiation reached new levels in 2017. *Food Safety News.* Retrieved from https://www.foodsafetynews.com/2018/01/acceptance-use-of-food-irradiation-reached-new-levels-in-2017/.

Falagán, N., & Terry, L. A. (2018). Recent Advances in Controlled and Modified Atmosphere of Fresh Produce. *Johnson Matthey Technology Review, 62*(1), 107-117. https://doi.org/10.1595/205651318X696684.

Fan, X., & Mattheis, J. P. (2001). 1-Methylcyclopropene and storage temperature influence responses of 'Gala' apple fruit to gamma irradiation. *Postharvest biology and technology, 23*(2), 143-151. https://doi.org/10.1016/S0925-5214(01)00119-3.

Fan, X., Thayer, D.W. (2001). Quality of irradiated alfalfa sprouts. *Journal of Food Protection*. 64(10), 1574-1578. https://doi.org/10.4315/0362-028x-64.10.1574.

Irradiation in the production, processing, and handling of food 21 CFR Part 179, (2019).

FGN. (2020). California pear growers come out strong against ripening treatments. Retrieved from https://fruitgrowersnews.com/news/california-pear-growers-come-out-strong-against-ripening-treatments/

Flaherty, E. J., DeEll, J. R., Shelp, B. J., & Bozzo, G. G. (2018). 1-Methylcyclopropene affects the shelf-life quality of controlled atmosphere stored 'Cold Snap™' pears. *Canadian journal of plant science, 98*(6), 1365-1375. https://doi.org/10.1139/cjps-2018-0136.

Flaherty, E. J., Lum, G. B., DeEll, J. R., Subedi, S., Shelp, B. J., & Bozzo, G. G. (2018). Metabolic Alterations in Postharvest Pear Fruit As Influenced by 1-Methylcyclopropene and Controlled Atmosphere Storage. *J. Agric. Food Chem, 66*(49), 12989-12999. https://doi.org/10.1021/acs.jafc.8b04912.

Follett, P. A., & Wall, M. M. (2013). Phytosanitary irradiation for export of fresh produce: commercial adoption in Hawaii and current issues. *Journal of Radioanalytical and Nuclear Chemistry, 296*(1), 517-522. https://doi.org/10.1007/s10967-012-1970-0.

Frylinck, L., Dubery, I. A., & Schabort, J. C. (1987). Biochemical changes involved in stress response and ripening behaviour of γ-irradiated mango fruit. *Phytochemistry (Oxford), 26*(3), 684-686. https://doi.org/10.1016/S0031-9422(00)84765-2.

Gapper, N. E., Bai, J., & Whitaker, B. D. (2006). Inhibition of ethylene-induced α-farnesene synthase gene Pc AFS1 expression in 'd'Anjou' pears with 1-MCP reduces synthesis and oxidation of α-farnesene and delays development of superficial scald. *Postharvest biology and technology, 41*(3), 225-233. https://doi.org/10.1016/j.postharvbio.2006.04.014.

Geweely, N., & Nawar, L. (2006). Sensitivity to Gamma Irradiation of Post-harvest Pathogens of Pear. *International Journal of Agriculture & Biology, 8*(6), 710-716.

Giné-Bordonaba, J., Busatto, N., Larrigaudière, C., Lindo-Garcia, V., Echeverria, G., Vrhovsek, U., Farneti, B., Biasioli, F., De Quattro, C., Rossato, M., Delledonne, M., Costa, F. (2020) Investigation of the transcriptomic and metabolic changes associated with superficial scald physiology impaired by lovastatin and 1-methylcyclopropene in pear fruit (cv. "Blanquilla"). *Horticulture Research*. 7, 49. https://doi.org/10.1038/s41438-020-0272-x.

GMA. (2009). Food Irradiation: A guide for consumers, policymakers, and the media. Retrieved from https://www.scantechsciences.com/wp-content/uploads/2017/03/GMA-Science-Policy-Paper-2009.Irradiation.pdf.

Gunes, G., Watkins, C. B., & Hotchkiss, J. H. (2000). Effects of irradiation on respiration and ethylene production of apple slices. *Journal of the science of food and agriculture, 80*(8), 1169.

Hallman, G. (2011). Phytosanitary Applications of Irradiation. *Comprehensive reviews in food science and food safety, 10*(2), 143-151. https://doi.org/10.1111/j.1541-4337.2010.00144.x.

Hallman, G. J. (2013). Control of stored product pests by ionizing radiation. *Journal of stored products research, 52*, 36-41. doi:10.1016/j.jspr.2012.10.001.

Hallman, G. J., & Blackburn, C. M. (2016). Phytosanitary Irradiation. *Foods, 5*(1), 10. https://doi.org/10.3390/foods5010008.

Hallman, G. J., & Loaharanu, P. (2016). Phytosanitary irradiation – Development and application. *Radiation Physics and Chemistry, 129*, 39-45. https://doi.org/10.1016/j.radphyschem.2016.08.003.

Hans, P. J. d. W., Els, C. O., & Herman, W. P. (2003). Carbon dioxide action on ethylene biosynthesis of preclimacteric and climacteric pear fruit. *J Exp Bot, 54*(387), 1537-1544. https://doi.org/10.1093/jxb/erg159.

Hansen, E., & Mellenthin, W. (1979). Commercial handling and storage practices for winter pears. In O. S. University (Ed.). Oregon State University.

Harder, M.N.C, Arthur, V., Arthur, P.B. (2016). Irradiation of foods: processing technology and effects on nutrients: effect of ionizing radiation on food components. *Encyclopedia of Food and Health*. 476-481. https://doi.org/10.1016/B978-0-12-384947-2.00405-0.

Hiwasa, K., Kinugasa, Y., Amano, S., Hashimoto, A., Nakano, R., Inaba, A., Kubo, Y. (2003). Ethylene is required for both the initiation and progression of softening in pear (*Pyrus communis* L.) fruit. *Journal of Experimental Botany*. 54(383), 771-779. https://doi.org/10.1093/jxb/erg073.

Hussain, P. R., Meena, R. S., Dar, M. A., & Wani, A. M. (2010). Carboxymethyl Cellulose Coating and Low-Dose Gamma Irradiation Improves Storage Quality and Shelf Life of Pear (Pyrus Communis L., Cv. Bartlett/William). *J Food Sci, 75*(9), M586-M596. https://doi.org/10.1111/j.1750-3841.2010.01868.x.

Igamberdiev, A.U., Eprintsev, A.T. (2016). Organic acids: the pools of fixed carbon involved in redox regulation and energy balance in higher plants. Frontiers in Plant Science. https://doi.org/10.3389/fpls.2016.01042.

Isidoro, N., & Almeida, D. P. F. (2006). α-Farnesene, conjugated trienols, and superficial scald in 'Rocha' pear as affected by 1-methylcyclopropene and diphenylamine. *Postharvest biology and technology, 42*(1), 49-56. https://doi.org/10.1016/j.postharvbio.2006.05.003.

Jemric, T., Lurie, S., Dumija, L., Pavicic, N., & Hribar, J. (2006). Heat treatment and harvest date interact in their effect on superficial scald of 'Granny Smith' apple. *Scientia horticulturae, 107*(2), 155-163. https://doi.org/10.1016/j.scienta.2005.07.001.

Jenjob, A., Uthairatanakij, A., Jitareerat, P., Wongs-Aree, C., & Aiamla-Or, S. (2017). Effect of harvest seasonal and gamma irradiation on the physicochemical changes in pineapple fruit cv. Pattavia during stimulated sea shipment. *Food Sci Nutr, 5*(5), 997-1003. https://doi.org/10.1002/fsn3.485

Jeong, M. A., & Jeong, R. D. (2018). Applications of ionizing radiation for the control of postharvest diseases in fresh produce: recent advances. *Plant pathology, 67*(1), 18-29. https://doi.org/10.1111/ppa.12739.

Jeong, R., Jeong, M., & Park, M. (2017). Gamma irradiation-induced disease resistance of pear (Pyrus pyrifolia "Niitaka") against Penicillium expansum. *Journal of phytopathology, 165*(9), 626-633. https://doi.org/10.1111/jph.12601.

Ju, Z., & Bramlage, W. J. (2000). Cuticular Phenolics and Scald Development in `Delicious' Apples. *Journal of the American Society for Horticultural Science, 125*(4), 498-504. https://doi.org/10.21273/JASHS.125.4.498.

Ju, Z., & Curry, E. A. (2000). Evidence that α-farnesene biosynthesis during fruit ripening is mediated by ethylene regulated gene expression in apples. *Postharvest biology and technology, 19*(1), 9-16. https://doi.org/10.1016/s0925-5214(00)00078-8.

Ju, Z., Curry, E. A., Duan, Y., Ju, Z., & Guo, A. (2001). Plant Oil Emulsions Prevent Senescent Scald and Core Breakdown and Reduce Fungal Decay in `Bartlett' Pears. *Journal of the American Society for Horticultural Science, 126*(3), 358-363. https://doi.org/10.21273/JASHS.126.3.358.

Kader, A. (1986). Potential Applications of Ionizing-Radiation in Postharvest Handling of Fresh Fruits and Vegetables. *Food Technology, 40*(6), 117-121.

Kader, A., & Mitcham, E. (2002). Postharvest Technology of Horticultural Crops In A. Kader (Ed.), *Postharvest Technology of Horticultural Crops* (3rd ed.). Davis, CA: UC Davis.

Kang, F., Hallman, G., Wei, Y., Zhang, F., & Li, Z. (2012). Effect of x-ray irradiation on the physical and chemical quality of America red globe grape. *African Journal of Biotechnology, 11*(31): 7966-7972. https://doi.org/10.5897/AJB11.4291.

Kaur, K., Dhillon, W.S. (2015). Influence of maturity and storage period on physical and biochemical characteristics of pear during post cold storage at ambient conditions. *Journal of Food Science and Technology*. 52, 5352–5356. https://doi.org/10.1007/s13197-014-1620-3.

Kheshti, N., Melo, A.A.M., Cedeno, A.B., Obenland, D., Prakash, A. (2019). Physiological response of 'Fuji' apples to irradiation and the effect on quality. *Radiation Physics and Chemistry*. 165, 108389. https://doi.org/10.1016/j.radphyschem.2019.108389.

Lindo-García, V., Giné-Bordonaba, J., Vall-Llaura, N., Duaigües, E., Larrigaudière, C. (2021). Unravelling the cold-induced regulation of ethylene and α-farnesene and its involvement with the development of scald-like disorders in different pear cultivars. *Postharvest Biology and Technology*. 174, 111441. https://doi.org/10.1016/j.postharvbio.2020.111441.

Lloyd-Jones, G. (2016). Basics of x-ray physics. Retrieved from https://www.radiologymasterclass.co.uk/tutorials/physics/x-ray physics production

Loayza, F.E., Tu, M., Prakash, A. (2020). Effectiveness of gamma irradiation in preventing postharvest defects in 'Granny Smith' apples.

Lu, J.Y., Miller, P., Loretan, P.A. (1989). Gamma radiation dose rate and sweet potato quality. *Journal of Food Quality*. 12(5), 369-376. https://doi.org/10.1111/j.1745-4557.1989.tb00337.x.

Lum, G. B., Deell, J. R., Hoover, G. J., Subedi, S., Shep, B. J., & Bozzo, G. G. (2017). 1-Methylcylopropene and controlled atmosphere modulate oxidative stress metabolism and reduce senescence-related disorders in stored pear fruit. *Postharvest Biology and Technology, 129*, 52-63. https://doi.org/10.1016/j.postharvbio.2017.03.008.

Lurie, S., & Watkins, C. B. (2012). Superficial scald, its etiology and control. *Postharvest Biology and Technology, 65*, 44-60. https://doi.org/10.1016/j.postharvbio.2011.11.001.

López, M. L., & Riba, M. (1999). Residue Levels of Ethoxyquin, Imazalil, and Iprodione in Pears under Cold-Storage Conditions. *J. Agric. Food Chem, 47*(8), 3228-3236. https://doi.org/10.1021/jf990117a.

Mabuchi, T., Matsumura, S. (1964). Dose rate dependence of mutation rates from γ-irradiated pollen grains of maize. *The Japanese Journal of Genetics*. 39(2-3), 131–135. https://doi.org/10.1266/jjg.39.131.

Mahto, R., & Das, M. (2013). Effect of gamma irradiation on the physico-chemical and visual properties of mango (Mangifera indica L.), cv. 'Dushehri' and 'Fazli' stored at 20°C. *Postharvest Biology and Technology, 86*, 447-455. https://doi.org/10.1016/j.postharvbio.2013.07.018.

Makkumrai, W., Anthon, G. E., Sivertsen, H., Ebeler, S. E., Negre-Zakharov, F., Barrett, D. M., & Mitcham, E. J. (2014). Effect of ethylene and temperature conditioning on sensory attributes and chemical composition of 'Bartlett' pears. *Postharvest Biology and Technology, 97*, 44-61. https://doi.org/10.1016/j.postharvbio.2014.06.001.

Marcu, D., Damian, G., Cosma, C., Cristea, V. (2013). Gamma radiation effects on seed germination, growth and pigment content, and ESR study of induced free radicals in maize *(Zea mays*). *Journal of Biological Physics*. 39, 625-634. https://doi.org/10.1007/s10867-013-9322-z.

Massey, L. M., Parsons, G. F., & Smock, R. M. (1964). Radiation Processing of Foods, Some Effects of Gamma Radiation on Keeping Quality of Apples. *J. Agric. Food Chem, 12*(3), 268-274. https://doi.org/10.1021/jf60133a025.

Maxie, E., Sommer, N., Muller, C., & Rae, H. (1966). Effect of Gamma Radiation on the Ripening of Bartlett Pears. *Plant Physiol, 41*(3), 437-442. https://doi.org/10.1104/pp.41.3.437.

McClain, B. (2019). [Phone Conference with Bob McClain/Interviewer: M. Tu & A. Prakash].

McHugh, T., & Liang, P. S. (2019). Realizing the Benefits of Food Irradiation. *Food Technology, 73*(9), 63-65.

Melo, A.A.M., Olabode, P.N., Atamian, H.S., Nyakundi, B., Toto-Pacioles, C., Prakash, A. (2021). Irradiation reduces superficial scald by downregulating ethylene and α-farnesene biosynthetic enzymes in 'Granny Smith' apples. *Radiation Physics and Chemistry*. 189, 109650. https://doi.org/10.1016/j.radphyschem.2021.109650.

Mielke, E. A., & Drake, S. R. (2004). Control of Storage-related Physiological Disorders of "d'Anjou" Pears by Integrated Reduced Dosage of Ethoxyquin and Low Oxygen Treatments. *Journal of food quality, 27*(6), 471-482. https://doi.org/10.1111/j.1745-4557.2004.00630.x.

Mitcham, E. (1996). Pear (Bartlett) Recommendations for Maintaining Postharvest Quality. Retrieved from http://postharvest.ucdavis.edu/files/259434.pdf.

Mitcham, E., Crisosto, C., & Kader, A. (1996). Pear (Bartlett Pears) Recommendations for Maintaining Postharvest Quality. Retrieved from http://postharvest.ucdavis.edu/files/259434.pdf.

Moggia, C., Moya-León, M. A., Pereira, M., Yuri, J. A., & Lobos, G. A. (2010). Effect of DPA [Diphenylamine] and 1-MCP [1-methylcyclopropene] on chemical compounds related to superficial scald of Granny Smith apples. *Spanish journal of agricultural research : SJAR, 8*(1), 178-187. https://doi.org/10.5424/sjar/2010081-1157.

Nath, A., Deka, B.C., Singh, A., Patel, R.K., Paul, D., Misra, L.K., Ojha, H. (2012). Extension of shelf life of pear fruits using different packaging materials. *Journal of Food Science and Technology*. 49, 556–563. https://doi.org/10.1007/s13197-011-0305-4.

Nguyen, T.T., Uthairatanakij, A., Srilaong, V., Laohakunjit, N., Kato, M., Jitareerat, P. (2021). Impact of electron beam irradiation on the chlorophyll degradation and antioxidant capacity of mango fruit. *Applied Biological Chemistry*. 64, 19. https://doi.org/10.1186/s13765-021-00592-8.

Nham, N. T., de Freitas, S. T., Macnish, A. J., Carr, K. M., Kietikul, T., Guilatco, A. J., . . . Mitcham, E. J. (2015). A transcriptome approach towards understanding the development of ripening capacity in 'Bartlett' pears (Pyrus communis L.). *BMC Genomics, 16*(1), 762. https://doi.org/10.1186/s12864-015-1939-9.

Nielsen, S. (2010). *Food analysis laboratory manual* (2nd Ed. ed.). New York, NY: Springer Science+Business Media, LLC

Nishiyama, I., Ikushima, T., Ichikawa, S. (1966). Radiobiological studies in plants—XI: Further studies on somatic mutations induced by x-rays at the al locus of diploid oats. Radiation Botany. 6(3), 211-218. https://doi.org/10.1016/S0033-7560(66)80057-1.

Nyakundi, B., Prakash, A., Atamian, H., & Olabode, P. (2019). Effect of Low Dose Irradiation on Expression of Genes Involved in Ethylene Biosynthesis of 'granny Smith' Apples. *Hortscience, 54*(9), S278-S278.

OSU. (2020). Pear (P*yrus* spp.) – Storage Rots. *Pest Management Handbooks.* Retrieved from https://pnwhandbooks.org/plantdisease/host-disease/pear-pyrus-spp-storage-rots

Pareek, S. (2019). *Postharvest physiological disorders in fruits and vegetables*. Boca Raton, FL: CRC Press.

Paul, V., & Pandey, R. (2014). Role of internal atmosphere on fruit ripening and storability—a review. *J Food Sci Technol, 51*(7), 1223-1250. https://doi.org/10.1007/s13197-011-0583-x.

Pechous, S., & Whitaker, W. (2004). Cloning and Functional Expression of an (E, E)-α-farnesene Synthase CDNA from Peel Tissue of Apple Fruit. *Planta, 219*(1), 84-94.

Pechous, S. W., Watkins, C. B., & Whitaker, B. D. (2005). Expression of α-farnesene synthase gene AFS1 in relation to levels of α-farnesene and conjugated trienols in peel tissue of scald-susceptible 'Law Rome' and scald-resistant 'Idared' apple fruit. *Postharvest biology and technology, 35*(2), 125-132. https://doi.org/10.1016/j.postharvbio.2004.08.005

Pedreschi, R., Franck, C., Lammertyn, J., Erban, A., Kopka, J., Hertog, M., . . . Nicolaï, B. (2009). Metabolic profiling of 'Conference' pears under low oxygen stress. *Postharvest biology and technology, 51*(2), 123-130. https://doi.org/10.1016/j.postharvbio.2008.05.019

Pedreschi, R., Hertog, M., Robben, J., Noben, J.-P., & Nicolaï, B. (2008). Physiological implications of controlled atmosphere storage of 'Conference' pears (Pyrus communis L.): A proteomic approach. *Postharvest biology and technology, 50*(2-3), 110-116. https://doi.org/10.1016/j.postharvbio.2008.04.004.

Pedreschi, R., Vanstreels, E., Carpentier, S., Hertog, M., Lammertyn, J., Robben, J., . . . Nicolai, B. M. (2007). Proteomic analysis of core breakdown disorder in Conference pears (Pyrus communis L.). *Proteomics, 7*(12), 2083-2099. https://doi.org/10.1002/pmic.200600723.

Pillai, S. (2016). Introduction to Electron-Beam Food Irradiation. Retrieved from https://www.aiche.org/resources/publications/cep/2016/november/introduction-electron-beam-food-irradiation

Pillai, S., Shayanfar, S., & Bolumar, T. (2015). *Electron beam pasteurization and complementary food processing technologies*. Cambridge, England: Cambridge, England : Woodhead Publishing.

Pintó, E., Lentheric, I., Vendrell, M., & Larrigaudière, C. (2001). Role of fermentative and antioxidant metabolisms in the induction of core browning in controlled-atmosphere stored pears. *Journal of the Science of Food and Agriculture, 81*(3), 364-370. https://doi.org/10.1002/1097-0010(200102)81:3<364::AID-JSFA828>3.0.CO;2-N.

Prakash, A., & Ornelas-Paz, J. J. (2019). Irradiation of fruits and vegetables. In E. Yahia (Ed.), *Postharvest Technology of Perishable Horticultural Commodities*. Cambridge, UK: Woodhead Publishing, Elsevier.

Rao, M.V., Watkins, C.B., Brown, S.K., Weeden, N.F. (1998). Active oxygen species metabolism in `White Angel' × `Rome Beauty' apple selections resistant and susceptible to superficial scald. *Journal of the American Society for Horticultural Science*. 123(2), 299–304. https://doi.org/10.21273/jashs.123.2.299.

Reisz, J.A., Bansal, N., Qian, J., Zhao, W., Furdui, C.M. (2014). Effects of ionizing radiation on biological molecules—mechanisms of damage and emerging methods of detection. *Antioxidants & Redox Signaling*. 21(2), 260–292. https://doi.org/10.1089/ars.2013.5489.

Reyes, L. F., & Cisneros-Zevallos, L. (2007). Electron-Beam Ionizing Radiation Stress Effects on Mango Fruit (Mangifera indica L.) Antioxidant Constituents before and during Postharvest Storage. *J. Agric. Food Chem, 55*(15), 6132-6139. https://doi.org/10.1021/jf0635661.

Rodríguez-Gómez, R., Vandeput, M., Zafra-Gómez, A., & Kauffmann, J.-M. (2018). Liquid chromatography-electrochemical detection for the determination of ethoxyquin and its dimer in pear skin and salmon samples. *Talanta, 177*, 157-162. https://doi.org/10.1016/j.talanta.2017.08.078.

Rupasinghe, H.P.V., Almquist, K.C., Paliyath, G., Murr, D.P. (2001). Cloning of hmg1 and hmg2 cDNAs encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase and their expression and activity in relation to α-farnesene synthesis in apple. *Plant Physiology and Biochemistry*. 39(11), 933–947. https://doi.org/10.1016/s0981-9428(01)01316-x.

Rupasinghe, H.P.V., Paliyath, G., Murr, D.P. (1998). Biosynthesis of α-farnesene and its relation to superficial scald development in `Delicious' Apples. *Journal of the American Society for Horticultural Science*. 123(5), 882–886. https://doi.org/10.21273/jashs.123.5.882.

Saltveit, M. (2012). Measuring respiration. Retrieved from http://ucce.ucdavis.edu/files/datastore/234-20.pdf.

Saltveit, M. E. (1999). Effect of ethylene on quality of fresh fruits and vegetables. *Postharvest Biology and Technology, 15*(3), 279-292. https://doi.org/10.1016/S0925-5214(98)00091-X.
Saquet, A. A. (2019). Storage of pears. *Scientia Horticulturae, 246*, 1009-1016. https://doi.org/10.1016/j.scienta.2018.11.091.
Sattar, A., Ali, M., Khan, I., & Muhammed, A. (1971). Effect of gamma radiation on post-harvest behaviour of pears. *Science and Industry, 8*(3/4), 330-333.
Sea, S., Rakovski, C., & Prakash, A. (2015). Ripening Quality of 'Bartlett' Pears (Pyrus communis L.) Subjected to Phytosanitary X-ray Irradiation Treatment Followed by Simulated Retail Display. *Hortscience, 50*(2), 279-287. https://doi.org/10.21273/hortsci.50.2.279.
Singh, S.P., Pal, R.K. (2009). Ionizing radiation treatment to improve postharvest life and maintain quality of fresh guava fruit. *Radiation Physics and Chemistry*. 78(2), 135–140. https://doi.org/10.1016/j.radphyschem.2008.09.007.
Tonetto de Freitas, S., & Pareek, S. (2019). *Postharvest physiological disorders in fruits and vegetables*. Boca Raton, FL: CRC Press, Taylor & Francis Group.
Tu, M. (2020). *Phytosanitary irradiation effects on the gene expression of ethylene forming enzymes and their role in superficial scald development in Bartlett pears.* (Master of Science in Food Science). Chapman University Chapman University.
UCD. (1971). Pear: Core Breakdown. *Fruit Physiological Disorders.* Retrieved from http://postharvest.ucdavis.edu/Commodity_Resources/Fruit_Physiological_Disorders/?uid=10&ds=822.
UCD. (2020). Pear: Senescent Scald. *Fruit Physiological Disorders.* Retrieved from http://postharvest.ucdavis.edu/Commodity_Resources/Fruit_Physiological_Disorders/?uid=13&ds=822.
USAP. (2020). Pear Varieties - Bartlett. Retrieved from https://usapears.org/bartlett/.
USDA. (2020). *Agriculture and Food Research Initiative Competitive Grants Program*. United States Department of Agriculture Retrieved from https://nifa.usda.gov/sites/default/files/rfa/FY2020-AFRI-Foundational-and-Applied-Science-RFA.pdf.
Villalobos-Acuña, M., & Mitcham, E. J. (2008). Ripening of European pears: The chilling dilemma. *Postharvest biology and technology, 49*(2), 187-200. https://doi.org/10.1016/j.postharvbio.2008.03.003.
Villalobos-Acuña, M. G., Biasi, W. V., Flores, S., Jiang, C.-Z., Reid, M. S., Willits, N. H., & Mitcham, E. J. (2011). Effect of maturity and cold storage on ethylene biosynthesis and ripening in 'Bartlett' pears treated after harvest with 1-MCP. *Postharvest biology and technology, 59*(1), 1-9. https://doi.org/10.1016/j.postharvbio.2010.08.001.
Watkins, C. (2009). Current and future research and uses of 1-MCP in apples. *Biology*, 14-26.
Wang, Y. (2016). Storage temperature, controlled atmosphere, and 1-methylcyclopropene effects on α-farnesene, conjugated trienols, and peroxidation in relation with superficial scald, pithy brown core, and fruit quality of 'd'Anjou' pears during long-term storage. *Journal of the American Society for Horticultural Science*. 141(2), 177–185. https://doi.org/10.21273/jashs.141.2.177.
Wani, A.M., Hussain, P.R., Meena, R.S., Dar, M.A. (2008). Effect of gamma-irradiation and refrigerated storage on the improvement of quality and shelf life of pear (*Pyrus communis* L., Cv. Bartlett/William). *Radiation Physics and Chemistry*. 77, 983-989. https://doi.org/10.1016/j.radphyschem.2008.04.005.
Whitaker, B. D., Villalobos-Acuña, M., Mitcham, E. J., & Mattheis, J. P. (2009). Superficial scald susceptibility and α-farnesene metabolism in 'Bartlett' pears grown in California and Washington. *Postharvest biology and technology, 53*(1-2), 43-50. https://doi.org/10.1016/j.postharvbio.2009.04.002.
WHO. (1988). *Food irradiation, a technique for preserving and improving the safety of food*. Retrieved from Geneva, Switzerland:

Yamaguchi, H., Shimizu, A., Degi, K., Morishita, T. (2008). Effects of dose and dose rate of gamma ray irradiation on mutation induction and nuclear DNA content in chrysanthemum. *Breeding Science*. 58, 331–335. https://doi.org/10.1270/jsbbs.58.331.

Yu, J., & Wang, Y. (2017). The combination of ethoxyquin, 1-methylcyclopropene and ethylene treatments controls superficial scald of 'd'Anjou' pears with recovery of ripening capacity after long-term controlled atmosphere storage. *Postharvest biology and technology, 127*, 53-59. https://doi.org/10.1016/j.postharvbio.2017.01.012.

Zdunek, A., Kozioł, A., Cybulska, J., Lekka, M., Pieczywek, P.M. (2016). The stiffening of the cell walls observed during physiological softening of pears. *Planta*. 243, 519-529. https://doi.org/10.1007/s00425-015-2423-0.

Zhao, J., Xie, X. B., Dai, W. H., Zhang, L. Z., Wang, Y., & Fang, C. B. (2018). Effects of precooling time and 1-MCP treatment on 'Bartlett' fruit quality during the cold storage. *Scientia Horticulturae, 240*, 387-396. https://doi.org/10.1016/j.scienta.2018.06.049.

Zhi, H., Dong, Y., & Wang, Y. (2019). Effects of controlled atmosphere, edible coating, and 1-methylcyclopropene in improving storage quality of 'Bartlett' pears after long-term storage. *Journal of Horticultural Science & Biotechnology, 94*(1), 94-101.

Zlatić, E., Zadnik, V., Fellman, J., Demšar, L., Hribar, J., Čejić, Ž., & Vidrih, R. (2016). Comparative analysis of aroma compounds in 'Bartlett' pear in relation to harvest date, storage conditions, and shelf-life. *Postharvest biology and technology, 117*, 71-80. https://doi.org/10.1016/j.postharvbio.2016.02.004.