Exploring the potential of low dose irradiation phytosanitary treatments for the Australian citrus industry

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Executive Summary

This review examined the published literature on the effects of low dose irradiation (< 1,000 Gy) as a market access treatment on citrus fruit quality. In summary the results highlighted:

- Postharvest low dose irradiation is an accepted safe phytosanitary treatment for some key export markets.
- Irradiation overcomes any issues of chilling injury with cold treatment for export.
- There are few consistent negative effects of irradiation on final citrus fruit quality following treatment and storage.
- Some surface damage (such as pitting) can occur in some types of different citrus but the contributing factors to this damage are not known but could potentially be managed.

Recommendations

Low dose irradiation provides market access options for the Australian citrus industry, but requires solutions to ensure consistent treatment out-turns which do not affect final fruit quality. Recommendations for future R&D investments include:

- Identify and manage pre- and postharvest factors (such as cultivar, harvest time, postharvest but pre-irradiation treatments) that contribute to potential post-treatment damage.

- Lemons and a lesser extent mandarins should be the focus of future industry R&D investments.

Contents

Executive Summary....1
Glossary....2
Introduction....3
Internal quality....3
Orange....4
Mandarin....4
Other citrus....5
Injury and disorders....5
Orange....8
Mandarin....10
Other citrus....11
Nutrition....12
Vitamin C....13
Orange....14
Mandarin....14
Other citrus....14
Other non-vitamin bioactive compounds....15
Consumer acceptability....17
Observations and opportunities....19
Conclusions....19
Recommendations....20
Acknowledgements....20
References....20

Glossary

Gy	Gray is a derived unit of ionizing radiation dose which is defined as the absorption of one joule of radiation energy per kilogram of matter. 1,000 Gy = 1 kGy
TA	Titratable acidity (% citric acid)
TSS	Total soluble solids, or soluble solids content (% Brix)
Vitamin C	Also known as ascorbic acid (AA) and is an essential nutrient in the diet

Introduction

Market access is the key priority of the Australian citrus industry. While cold treatment protocols have successfully enabled exports of oranges and mandarins to key export markets, the development of chilling injury in response to cold treatment can limit exports in some susceptible citrus types such as lemons. Another market access tool for the Australian citrus industry is low dose irradiation.

Irradiation is a technologically proven, viable and scientifically sound disinfestation treatment (Follet, 2009). Moreover, irradiation is increasingly becoming an approved and agreed treatment in world trade of food and horticultural products. Generic irradiation treatments have been approved by U.S. Department of Agriculture - Animal and Plant Health Inspection Service (USDA-APHIS) at doses of 150 Gy (Gray) for tephritid fruit flies and 400 Gy for all insects except pupal and adult Lepidoptera (USDA-APHIS, 2006). Further APHIS rulings and new rulings by the International Plant Protection Convention (IPPC) have approved new minimum doses for 6 fruit fly pests and 14 other plant insect pests regardless of the host product, at doses between 60 and 300 Gy (USDA-APHIS, 2008). An excellent review of irradiation quarantine treatments has been published by Follet (2009).

Food Standards Australia New Zealand (FSANZ) have granted food safety approval for irradiation (at 150 – 1,000 Gy) as a technique for insect pest disinfestation of citrus and it has also been approved in several export markets such as Indonesia and Vietnam. Irradiation breaks chemical bonds in DNA and other molecules, thereby sterilizing the pest or preventing it from achieving sexual maturity. This review examines the published literature on the effect of low dose irradiation (< 1,000 Gy) as a market access treatment on citrus fruit quality.

Internal quality

Total soluble solids (TSS) also known as soluble solids content (SSC, Brix %) is a relative measure of the sugar content in citrus fruit. Together with titratable acidity (TA) which is a measure of the acidity or sourness of the fruit, these fruit quality components combine to determine overall fruit taste and acceptability. The effects of low dose irradiation on TSS and TA levels in citrus fruit are presented in Table 1 and show that in general irradiation does not consistently affect TSS or TA in citrus fruit.

Table 1. Effects of irradiation on total soluble solids (TSS, % Brix) and titratable acidity (TA, % citric acid) in citrus fruit.

Fruit	Dose (Gy)	TSS	TA	Reference
Grapefruit Rio Red	70, 200, 300, 400, 700	Early season not affected. Later harvest no differences after storage	Early season not affected. Later harvest irradiated higher TA	Patil et al. (2004)
Grapefruit Rio Red	150, 300, 400, 500	No change	No change	Hallman and Martinez (2001)
Orange Navel	200, 400, 600	No difference, (except 400 and 600 Gy lower TSS)	No difference, (except at 600 Gy lower TA)	McDonald et al. (2013)
Orange Ambersweet,	250, 300, 450	No effect (except decrease in	No effect	Miller et al. (2000)

Fruit	Dose (Gy)	TSS	TA	Reference
Hamlin, Navel, Pineapple, Valencia		Ambersweet at 300 Gy)		
Mandarin Clementine	510, 875	No consistent differences	No consistent differences	Palou et al. (2007a)
Mandarin Clementine	195, 395, 510, and 875	No consistent differences	No consistent differences	Palou et al. (2007b)
Mandarin Fallglo, Minneola, Murcott, Sunburst, Temple	250, 300, 450	No effect (except decrease in Sunburst at 450Gy)	No effect (except decrease in Sunburst and Tangelo at 450Gy, and increase Murcott at 300Gy)	Miller et al. (2000)
Mandarin Seedless Kishu	150, 400, 1,000	No change	No change	Ornelas-Paz et al. (2017)
Mandarin Shatang	200, 300, 400, 500, 600	Up to 30 days storage - no differences	Decrease TA with increasing dose, during storage	Zhang et al. (2014)
Orange Navel	200, 400, 600, 800, 1,000	No change	No change	Noh et al. (2016 a, b)
Orange Marrs	150, 300, 400, 500	No change	No change	Hallman and Martinez (2001)
Lime Tahitian	50, 100, 150 and 200; 250, 750	Response varied with harvest time	Response varied with harvest time	da Silva et al (2016)
Pommelo Sarawak, Chandler	150, 1,000	No consistent effects	No consistent effects	Jain et al. (2017)

Orange

O'Mahony and Goldstein (1987) and McDonald et al. (2013) found a decrease in TSS and TA at irradiation dose levels between 300 and 600 Gy but Miller et al. (2000) did not report changes in TA of oranges treated at irradiation doses of 150 – 450 Gy. McDonald et al. (2013) concluded that the overall changes in combined TSS and TA as shown by changes in BrimA after 4 weeks of storage, were relatively small (0.31 units). This, combined with the results their sensory testing, suggested that the effects of irradiation on TSS and TA may have limited importance in terms of an impact on flavor.

In a study of low dose irradiation (200, 400, 600, 800 and 1,000 Gy) on imported Navel oranges stored for either 20ºC for 12 days or 3ºC for 45 days reported no significant effect of irradiation in TSS/TA ratio, total sugar content, reducing sugar content (Noh et al., 2016 a, b). While Hallman and Martinez (2001) found no change in TA or TSS in Marrs oranges treated up to 500 Gy and stored for 21 days at ambient temperature.

Mandarin

Miller et al. (2000) showed that irradiation (150, 300 and 450 Gy) did not generally affect TSS or TA of five mandarin hybrids, Fallglo, Minneola, Murcott, Sunburst, and Temple, with the exception of a decrease in of Sunburst mandarin at 450 Gy and a reduction in TA of Sunburst and Temple at 450 Gy, but an increase at 300 Gy in Murcott. While these few differences may have been statistically different, the effects on overall taste and flavour would be low. Indeed with a small seven person untrained panel, Miller et al. (2000) showed the acceptability of juice and pulp flavor was not affected. Sensory and consumer aspects of irradiation on consumer acceptability is discussed in the *Consumer Acceptability Section*. Ornelas-Paz et al. (2017) further showed no consistent effect of low dose irradiation (150, 400, and 1,000 Gy) on TSS or TA in Seedless Kishu mandarins. While Zhang et al. (2014) showed with Shatang mandarin treated at 200, 300, 400, 500 and 600 Gy, TSS and TA had no significant differences compare to those of the control during the first 2 weeks storage, but after this storage time at 4 °C, increasing dose reduced TA levels. However there were no significant differences in TSS between irradiated and non-treated fruits after 30 day storage (Zhang et al. 2014). Palou et al. (2007a, b) also showed there were no consistent differences in TSS and TA in Clemenules mandarins after different times in storage where in general, irradiation had no effect on TSS or TA.

Other citrus

Grapefruit

Patil et al. (2004) showed harvest time had a significant effect on the treatment effects of irradiation on Rio Red grapefruit. In early season grapefruit, TSS and TA were not affected due to irradiation or storage, but late harvest grapefruit exposed to irradiation (70 – 700 Gy) retained acidity better than the fruit not exposed to irradiation. Patil et al. (2004) further showed that the initial TSS was the lowest in the late season fruit exposed to the 700 Gy treatments; however, no differences among treatments were observed after storage. Hallman and Martinez (2001) further found no change in TA or TSS in Rio Red grapefruit treated up to 500 Gy and stored for 21 days at ambient temperature.

Lime

The effects of irradiation in the internal quality of Tahitian limes were affected by harvest time ('on'- and 'off'-season fruit) and irradiation dose (da Silva et al. 2016).They showed irradiation reduced TSS in fruit from the 'off'-season, but not fruit harvested in the regular harvest period. They also showed higher TA values in the 'off'-season fruits treated with 50 Gy, as compared with untreated fruits (da Silva et al. 2016). This in this trial, the interaction of harvest time and irradiation was significant and should be taken into account.

Pummelo

Jain et al. (2017) showed no consistent effects of 150 and 1,000 Gy irradiation on TSS and TA values in Chandler and Sarawak pummelos stored for 3 weeks at 12 °C and after an additional week at 20 °C. The differences were low and did not affect consumer acceptability of the treatment (Jain et al. 2017).

Injury and disorders

Citrus is relatively sensitive to irradiation and the response to treatment is highly variable and dependent on species, hybrid, and cultivar (Miller et al, 2000). Physical injury can occur at low doses (< 1,000 Gy) and usually occurs in the peel as peel pitting and fruit softening (Wall 2015). For example Miller et al. (2000) treated ten citrus cultivars grown in Florida, including the five orange [*Citrus sinensis* (L.) Osbeck] cultivars, Ambersweet, Hamlin, Navel, Pineapple, and Valencia, and the five mandarin hybrids (*Citrus reticulata* Blanco), Fallglo, Minneola, Murcott, Sunburst, and Temple, with low dose irradiation at 0, 250, 300 and 450 Gy, and stored for 14 days at 1 °C or 5 °C plus 3 days at 20 °C, to determine dose tolerance based on fruit injury. They showed that fruit softening of Valencia, Minneola, Murcott, and Temple was dose-dependent, but that of other cultivars was unaffected. Only Ambersweet, Valencia, Minneola, and Murcott did not develop peel pitting at 150 Gy or higher, whilst all the other cultivars were injured (Miller et al. 2000).

While limited studies have been conducted on the causes of damage, Riov (1975) showed that irradiation induced pitting which may be caused by the accumulation of phenolic compounds in flavedo cells leading to cell death and peel necrosis that is manifest as pitting (Figure 1).

A summary table of the literature on the effect of irradiation on citrus disorders in presented in Table 2, and shows different citrus types develop different types of symptoms at different treatment doses.

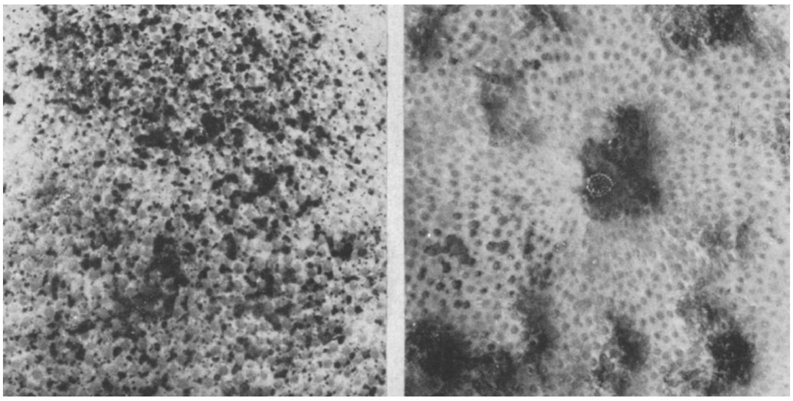


Figure 1. Symptoms of external radiation damage in Marsh Seedless grapefruit peel 7 days after irradiation with 2,400 Gy. Damage symptoms at the stem end (left) and damage symptoms at the stylar end (right).
From Riov (1975) Radiation Botany 15, 257-260.

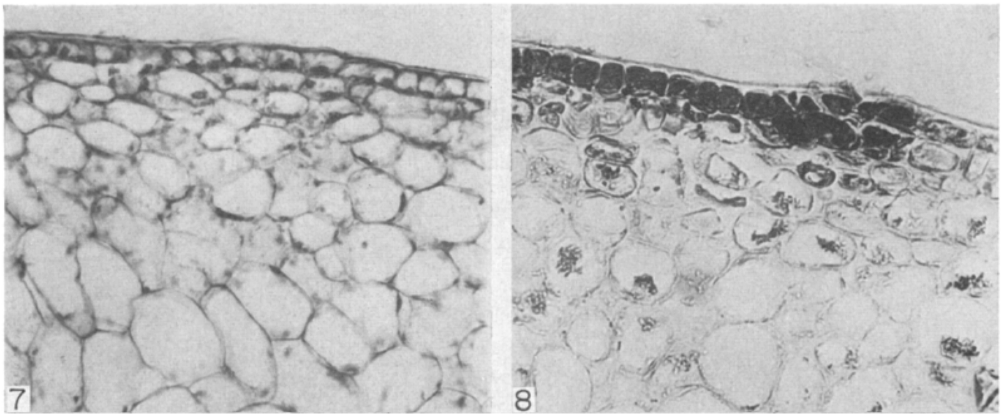


Figure 2. Cross sections in the outer flavedo layers at the stem end of control and irradiated Shamouti orange treated with 2,400 Gy. Untreated control Shamouti orange stained with diazo-safranin (× 290) (left). Irradiated Shamouti orange stained with the GIBBS' reagent (right). Phenolic compounds which accumulate in the outer flavedo layers are seen mainly in the epidermis (× 290).
From Riov (1975) Radiation Botany 15, 257-260.

Table 2. Effects of irradiation on visual quality of citrus fruit.

Fruit	Dose (Gy)	Injury / Disorder	Reference
Orange Mosambi	250, 500, 1,000, 1,500	Peel disorder in the form of brown sunken areas after 90 days and reduced fruit firmness at > 250 Gy	Ladaniya et al. (2003)
Orange Ambersweet, Hamlin, Navel, Pineapple, and Valencia	0, 250, 300, 450	Fruit softening of Valencia was dose-dependent, but that of all other cultivars was unaffected. Pitting - Hamlin, Navel, Pineapple developed peel pitting injury, but Ambersweet and Valencia did not develop any symptoms.	Miller et al. (2000)
Orange Lane Late	200, 400, 600	Surface pitting and visual damage after treatment with 400 and 600 Gy – see Figure 3	McDonald et al. (2013)
Orange Navel	320-370 and 520-600	Increased brown blemishing and pitting	O'Mahony and Goldstein (1987)
Orange Valencia	300, 500, 750, 1,000	No damage at 750 Gy	Nagai and Moy (1985)
Orange Washington Navel, Valencia	75, 150, 300	No damage up to 300 Gy	Macfarlane and Roberts (1968)
Orange Valencia Late	350, 800	No damage	Betancurt et al. (2007)
Mandarin Fallglo, Minneola, Murcott, Sunburst, Temple	0, 250, 300, 450	Fruit softening of Minneola, Murcott, and Temple was dose-dependent, but that of other cultivars was unaffected. Pitting - Minneola, and Murcott did not develop peel pitting at 150 Gy or higher, whilst all the other cultivars were injured	Miller et al. (2000)
Mandarin Nagpur	250, 500, 1,000, 1,500	No rind disorders up to 1,500 Gy	Ladaniya et al. (2003)
Mandarin Clemenules	195, 395	Slight to moderate rind browning immediately after treatment, but no damage after 12 days cold storage	Alonso et al. (2007)
Mandarin Seedless Kishu	150, 400, 1,000	400 and 1000 Gy promoted browning of the calyx end and fungal infection	Ornelas-Paz et al. (2017)
Mandarin Shatang	200, 300, 400, 500, 600	No effect, but increased decay with high levels	Zhang et al. (2014)
Grapefruit Rio Red	150, 300, 400, 500	No effect	Hallman and Martinez (2001)
Grapefruit Rio Red	70, 200, 400, 700	Early season Rio Red grapefruit was more sensitive to later season fruit, particularly at 700 Gy in the early season fruit. But overall appearance of all fruit was still acceptable as judged by consumers	Patil et al. (2014)

Fruit	Dose (Gy)	Injury / Disorder	Reference
Pumello Chandler, Sarawak	150, 1,000	Peel damage was greater and developed more quickly in irradiated fruit and was more severe when the fruit was stored at ambient temperature for a week	Jain et al. (2017)
Lemon Lisbon	75, 150, 300, 600, 1,000	Peel damage increased with dose. Lemons at a green stage were more severely affected than yellow lemons	Jessup et al. (1992)
Lemon Eureka	500 – 3,000	Some minor peel damage and cavitation at 500 Gy	Maxie et al. (1964)
Lime Tahitian	50, 100, 150 and 200; 250, 750	> 100 Gy caused skin yellowing	da Silva et al (2016)

Orange

Lane Late navel oranges had increased surface pitting and visual damage after treatment with 400 and 600 Gy (McDonald et al. 2013). Figure 3 shows the typical symptoms of irradiation damage, with brown blemish and pitting in Lane Late navel oranges following treatment at 400 and 600 Gy and storage. Table 3 quantifies the levels of damage in these fruit following treatment and storage. O'Mahony and Goldstein (1987) also found increased brown blemishing and pitting of whole navel oranges irradiated at 300 and 600 Gy. While in another study, Valencia oranges were tolerant to 750 Gy treatment and storage for 7 weeks at 7 °C (Nagai and Moy 1985). In a comparison of different orange cultivars, Miller et al. (2000) showed that Ambersweet and Valencia oranges tolerated 500 - 600 Gy irradiation, but Hamlin, Navel, and Pineapple cultivars were injured at 150 Gy.

Macfarlane and Roberts (1968) showed treating Washington Navel oranges with less than 300 Gy were commercially acceptable, however injury increased as the dose was increased over the range studied. In addition, the severity of injury depended on the variety and, particularly for Washington Navels, on the part of the season when the fruit was picked and treated (Macfarlane and Roberts, 1968). From a limited data set, early season Washington Navel fruit harvested from coastal NSW was very susceptible to injury, whilst late season fruit was relatively resistant. In a subsequent smaller experiment with Valencia oranges, time of harvesting made little difference (Macfarlane and Roberts 1968). Indeed Betancurt et al. (2007) also showed no effect of 350 and 800 Gy irradiation on Late Valencia appearance and fruit quality.

In addition to external appearance, McDonald et al. (2013) found detected some internal drying and granulation in Lane Late navel oranges with higher irradiation treatments. They showed that after 3 weeks of storage, 25% and 29% of the fruit treated at doses of 400 and600 Gy, respectively, showed some degree of segment drying, but none of the control or fruit treated at 200 Gy showed any symptoms. In addition, they showed granulation was present in an average of 17% of the 600 Gy fruit and in none of the other treatments. However the extensive taste panel assessment did not detect any differences changes due to dose or storage time (McDonald et al., 2013). However in other studies, with Clemenules mandarin fruit (Alonso et al., 2007), Fortune hybrid fruit Alonso et al. (2002) and Nagpur mandarins (Ladaniya et al. 2003) there has been no reports of juice loss due to irradiation.

Figure 3. Effect of low dose irradiation on visual quality of Lane Late navel oranges after 4 weeks storage at 3 °C (left) and 3 weeks at 3 °C and one week at 20 °C shelf-life (right). [*from McDonald et al. 2013*]

4 weeks at 3 °C 3 weeks at 3 °C + 1 week at 20 °C

Control

200 Gy

400 Gy

600 Gy

(from McDonald et al. (2013). Effect of gamma irradiation treatment at phytosanitary dose levels on the quality of 'Lane Late' navel oranges. Postharvest Biology and Technology 86, 91-99)

Table 3. External damage values for Lane Late navel oranges treated at four different dose levels and evaluated after 1 d, 3 weeks and 4 weeks following irradiation treatment with injury / damage scored using 1–6 scale where 1 = no damage, 4 = moderate damage, 6 = very severe damage. % of fruit rated moderate or above is indicated next to predicted mean score. *(from McDonald et al. 2013).*

	External damage score and % of fruit rated 4 (moderate) and above					
Dose (Gy)	1 day		3 weeks		4 weeks	
0	2.0	6.9%	2.1	6.6%	2.0	3.5%
200	1.9	4.5%	2.3	7.3%	2.4	11.1%
400	2.1	10.8%	3.0	33.0%	3.4	50.3%
600	2.1	9.4%	3.6	55.9%	3.8	68.4%

Mandarin

Similar to oranges, the tolerance of mandarins to irradiation is dependent on cultivar and other pre and postharvest factors. Miller et al. (2000) showed that the mandarin hybrids Minneola and Murcott showed tolerance at 500-600 Gy, but peel pitting occurred for Fallglo, Sunburst and Temple cultivars at 150 Gy. Ladaniya et al. (2003) showed the Nagpur mandarin tolerated doses up to 1,000 Gy. While Shatang mandarin was not affected by treatment up to 600 Gy (Zhang et al. 2014).

Alonso et al. (2007) observed a slight to moderate rind browning was observed in X-ray irradiated Clemenules mandarin fruit at both 195 and 395 Gy after two days at 20 °C. However, this damage was not evident after the 12-day cold storage period and this underscores the random nature of these disorders and could be attributed to interactions with other postharvest factors such as the coating used in this experiment (Alonso et al. 2007).

While some cultivars have tolerance to irradiation, some mandarin types are very sensitive to irradiation. For example, Ornelas-Paz et al. (2017) found Seedless Kishu mandarins (*Citrus kinokuni mukakukishu*) treated with 150 Gy developed peel damage and browning of the calyx. This is not the classic pitting damage, but a general browning and senescence of the peel which becomes highly susceptible to fungal damage (Figure 4). The severity of the browning damage increased with irradiation dose, especially for fruit located on the top layer of the cases but browning severity was not objectively evaluated in this study and further studies are needed in this regard.

Figure 4. Irradiation damage on the peel of kishu mandarins after three weeks of storage.

(from Ornelas-Paz et al. (2017) Effect of phytosanitary irradiation on the postharvest quality of Seedless Kishu mandarins (Citrus kinokuni mukakukishu). Food Chemistry 230, 712-720.)

Other citrus

Grapefruit

Treatment of grapefruit with a dose of 300 Gy resulted in minimal injury to the fruit (Spalding and Davis 1985; Miller and McDonald 1996). While Hallman and Martinez (2001) demonstrated that Rio Red grapefruit exposed to irradiation doses of up to 500 Gy did not affect appearance compared to untreated fruit. Type and intensity of injury to grapefruit due to low dose irradiation (300–900 Gy) has been attributed to time of harvest. Early-season grapefruit, harvested from October to December (northern hemisphere), were more susceptible to scald and less susceptible to rind breakdown, while late-season fruit were more susceptible to rind breakdown after irradiation and storage (Hatton et al., 1982). Patil et al. (2014) further showed early season Rio Red grapefruit was more sensitive to later season fruit, particularly at 700 Gy in the early season fruit, overall appearance of all fruit was still acceptable as judged by consumers.

Pummelo

There was peel damage in Chandler (red flesh) and Sarawak (white flesh) pummelos treated with 150 Gy and 1000 Gy is described in Figure 5 (Jain et al. 2017). The damage was greater and developed more quickly in irradiated pummelos and became even more severe when the fruit was stored at ambient temperature for a week. However this damage was low and Jain et al. (2017) suggest this could be managed with minimal handling and good temperature control. Indeed the quality of irradiated pummelos stored at refrigerated temperature for 3 weeks was similar to untreated pummelos, however, physical handling and exposure to higher temperature resulted in increased peel pitting of irradiated fruit compared to non-treated fruit. Jain et al (2017) conclude that irradiation could serve as a potential phytosanitary treatment for Chandler and Sarawak pummelos, provided that the fruit is subjected to minimal handling and not temperature abused.

Figure 5. Peel damage in 150 Gy Chandler pummelo after 4 weeks of storage - 3 weeks at 12 °C and fourth week at 20 °C.

(from Jain et al. (2017) Effect of phytosanitary irradiation on the quality of two varieties of pummelos (Citrus maxima (Burm.) Merr.). Scientia Horticulturae 217, 36-47)

Lemon

Lisbon lemons harvested at two different maturity stages, green and completely yellow which grown in the NSW Central Coast, were treated with 75, 150, 300, 600 and 1,000 Gy (Jessup et al. 1992). They showed that peel damage increased with increasing irradiation dose. Lemons that were harvested at a green stage, were more severely affected than yellow lemons (Table 4). In addition irradiation caused flesh discolouration and cavitation (Table 4), as well as albedo discolouration and toughness, particularly at the higher dose rates (Jessup et al. 1992).

Maxie et al. (1964) treated Eureka lemons with high irradiation treatments (500 – 3,000 Gy), but even at the lower irradiation doses (500 Gy), there was some minor peel damage and cavitation.

Table 4. Effect of low dose irradiation and subsequent storage at 15 °C on damage to Lisbon lemons harvested at either a green and completely yellow stage. (from Jessup et al. 1992)

Type of damage to fruit and maturity tested						
	Peel Damage		Flesh discolouration		Cavitation	
	Yellow	Green	Yellow	Green	Yellow	Green
5% lsd	*0.42*		*0.45*		*0.23*	
0	1.0	1.0	1.0	1.0	1.0	1.0
75	1.8	2.3	1.1	1.0	1.0	1.0
100	2.4	3.1	2.0	2.1	1.0	1.0
300	2.6	3.6	2.7	2.1	1.2	1.0
600	2.5	3.1	2.6	2.3	1.3	1.2
1000	3.0	4.0	3.5	2.5	1.7	1.2

Lime

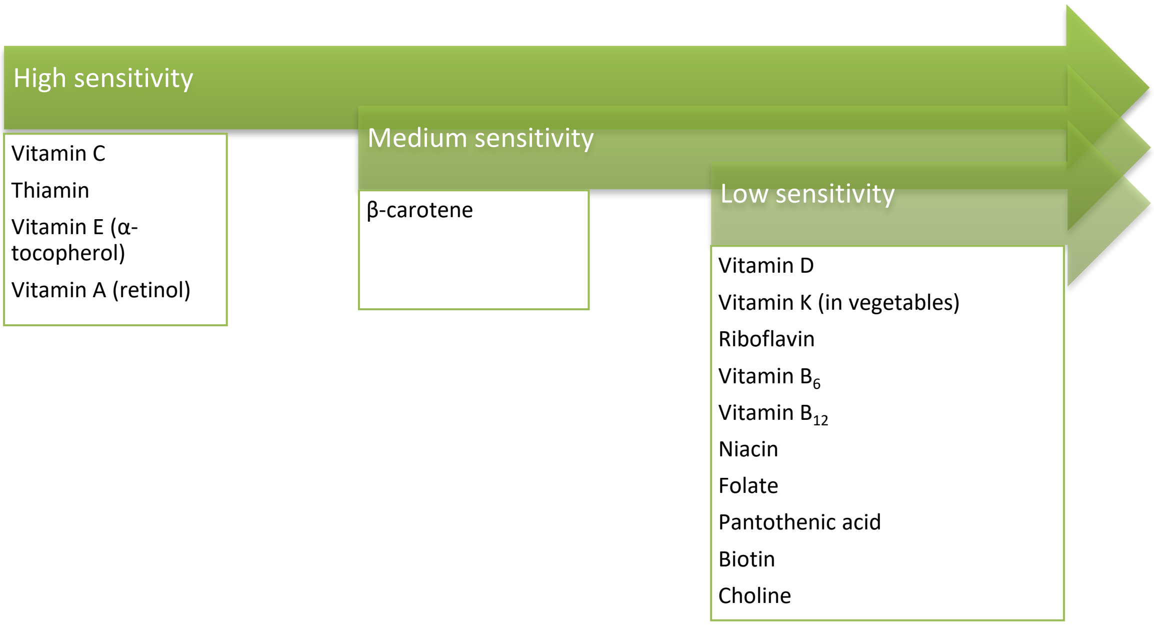
In a preliminary trial, da Silva et al (2016) showed 250 and 750 Gy doses negatively affected skin quality and pulp of Tahitian limes. They further used lower doses of irradiation (50, 100, 150, and 200 Gy) and showed doses > 100 Gy caused skin yellowing of fruits harvested and concluded doses between 50 Gy and 700 Gy caused damage to lime fruit quality during storage at room temperature (de Silva et al., 2016).

Nutrition

Numerous independent reviews have been published on the effects of irradiation on the nutritional quality of food, including fresh fruit and vegetables (World Health Organization 1981; World Health Organization 1994; World Health Organization 1999; Scientific Committee on Food 2003; Arvanitoyannis 2010; European Food Safety Authority 2011). These reviews have examined the efficacy, safety and nutritional effects of irradiation on a wide range of foods, including citrus. Irradiation can induce changes in nutrient content, depending on a variety of factors including the irradiation dose, composition of the food, packaging material, ambient temperature and atmospheric oxygen concentration (Diehl et al. 1991; Kilcast 1994; World Health Organization 1994). A relatively small proportion of nutrients are sensitive to irradiation, with higher doses of irradiation associated with greater nutritional losses (World Health Organization 1999), however these doses are more than the maximum 800 Gy used for disinfestation of fresh fruit and vegetables. Indeed, there has been no demonstrated effect of irradiation up to 1,000 Gy on the amount and nutritional quality of carbohydrates, proteins or fats and no evidence to suggest that irradiation reduces the mineral content of food (Diehl et al. 1991; World Health Organization 1994). Therefore, macronutrients and minerals have not been given further consideration in this review.

Vitamins have been shown to be susceptible to oxidation and breakdown with high levels of irradiation treatment. There is a general hierarchy of vitamin sensitivity to irradiation, with vitamins A, C, E and thiamin being most sensitive (Figure 6) (Kilcast 1994; Diehl 1995). As fruits and vegetables are the predominant dietary sources of vitamin A (as carotene) and vitamin C, the majority of studies examining the effects of irradiation on fruit or vegetable quality have focused on these nutrients.

Figure 6. General sensitivity of vitamins in fresh fruit and vegetables to irradiation.



(modified from FSANZ 2014 and Kilcast 1994)

Vitamin C

Citrus is a rich source of ascorbic acid (AA, vitamin C) and is a major dietary contributor to AA in all age and gender groups in Australia and New Zealand, with the exception of 17-18 year old Australian females (FSANZ 2014). Indeed, citrus fruit provides between 5 - 17% of AA intake of Australians (FSANZ 2014). Therefore the potential effects of irradiation of vitamin C content of citrus is important. Citrus are not a major source of dietary carotene, thiamin, riboflavin, niacin, folate or vitamins E and B6 in Australia and New Zealand (FSANZ 2014), therefore this review will focus on the effects of irradiation treatment on AA and carotenes.

Substantial data documents the natural variation in levels of vitamin C in citrus fruit (Lee and Kader 2000; Magwaza et al. 2017). Extensive natural variation occurs in the vitamin C content in of citrus where the main sources of variation are cultivar, season, growing location and orchard management, indeed there is more than four-fold being common between citrus types (Magwaza et al. 2017). In addition, postharvest treatments and storage result in decreased levels of vitamin C during storage (Lee and Kader 2000; Mditshwa et al. 2017).

Vitamin C is widely regarded as a most important water-soluble antioxidant. It includes all compounds exhibiting the biological activity of L-ascorbic acid (AA) plus L-dehydroascorbic acid (DHAA), its oxidation product (Lee and Kader, 2000). However vitamin C is inherently unstable in solution, with its destruction affected by temperature, light and pH (Eitenmiller et al. 2008). As such, vitamin C is one of the most sensitive vitamins to irradiation, with the effects of irradiation influenced by exposure to oxygen, storage and temperature, as well as the pH of the food matrix or storage medium (Kilcast 1994). Irradiation results in some AA being converted to DHAA (Kilcast 1994), however both forms have vitamin C activity (Tsujimura et al. 2008). Therefore, when interpreting findings of irradiation studies it is important to consider that losses due to irradiation may be overestimated if only AA is reported. Hence, total vitamin C (AA plus DHAA) content is a more reliable indicator of post-irradiation vitamin C.

Citrus fruit

There have been a number of studies on the effects of irradiation on nutrient composition of orange, mandarin, lemon, lime and grapefruit. The findings of these studies are summarised in Table 5 (FSANZ 2014) with additional data from the literature.

Orange

In Kau oranges, there was no significant effect of X-ray irradiation at 750 Gy on AA levels. Similarly, total carotenoids did not change with irradiation after two days, but were increased 33% after nine days storage in irradiated oranges (Boylston et al. 2002). In blood oranges, irradiation with 250 and 500 Gy slowed the loss of AA during six weeks storage, resulting in higher AA levels in oranges irradiated with 500 Gy (Khalil et al. 2009).

In Mosambi oranges, AA decreased initially at doses of 1,000 Gy (-22%) and 1,500 Gy (-16%). However, this effect was lost throughout the storage period as all groups exhibited AA losses (0 Gy; -31%. 250 Gy; -26%, 500 Gy; -33%, 1,000 Gy; -4%, 1,500 Gy; -16%) (Ladaniya et al. 2003). As only AA was measured, some of the variability in these data may be through transformation to DHAA. Furthermore, the statistical analyses were limited to ANOVA; results of post-hoc testing were not presented thereby limiting interpretation as dose-effects cannot be separated.

De Bortoli et al (2015) report that low dose irradiation (10, 20, 30, 40, 50, 100, 150 and 200 Gy) had no effect on AA levels in Valencia oranges, however no data or methodology were reported and little impact should be taken from this report.

Noh et al. (2016) and Cho et al. (2015) showed that in general treatment with 200, 400, 600, 800 and 1,000 Gy resulted in statistically lower vitamin C levels in Navel oranges imported into Korea during storage at 3°C and at room temperature (20°C) respectively, but these effects were variable at different storage times.

Mandarin

Irradiation with 75 and 300 Gy had no significant effect on total vitamin C content in Ellendale mandarins, within a week of irradiation or after three weeks storage (Mitchell et al. 1992). Vitamin C levels decreased 10-12% in Ellendale mandarins, irrespective of irradiation. In contrast, Imperial mandarins showed no early effects of irradiation, but after three weeks storage vitamin C levels decreased 46% in non-irradiated fruit and 69% and 78% in fruit irradiated at 75 and 300 Gy respectively. At this time, total vitamin C levels were significantly lower in irradiated compared to control mandarins.

Another study measured AA levels in Nagpur mandarins irradiated with 250, 500, 1,000 and 1,500 Gy. Irradiation doses of ≥ 500 Gy decreased AA content by approximately 15% (Ladaniya et al. 2003). However, diminution of AA was not dose-dependent, and AA levels fluctuated throughout the storage period. This variability in AA suggests conversion between AA and DHAA may be occurring. As DHAA was not measured, it is not possible to determine the extent of vitamin C loss in this study.

Clementine mandarin A study in Clementine mandarins detected no significant change in total vitamin C levels after X-ray irradiation with 510 and 875 Gy (Rojas-Argudo et al. 2012). Similarly, work from the same group using up to 164 Gy in combination with up to 12 days storage showed no significant change in total vitamin C, except for an early increase in total vitamin C in Clementine mandarins irradiated with 54 Gy (Contreras-Oliva et al. 2011). A third study in Clementine mandarins assessed the impact of irradiation in combination with washing / waxing and storage (Mahrouz et al. 2002). In this study, AA levels fluctuated throughout the seven week experimental period, but decreased in all groups during storage. After seven weeks, there was no significant effect of irradiation on AA content (Mahrouz et al. 2002).

Other citrus

Grapefruit

Two studies from the same group indicate no consistent effect of low dose irradiation on AA and β-carotene levels in grapefruit. In the first study, there was no effect of irradiation with 70 – 700 Gy on β-carotene or total carotenoid levels in Rio Red grapefruit in early harvest fruit after 35 days storage at 10C (Patil et al. 2004). However, irradiation doses of 200 Gy or higher, significantly reduced vitamin C content after 4 weeks of storage at 10 °C in late-season Rio Red grapefruit (Patil et al., 2004).

β-carotene levels increased with storage in early harvest fruit irrespective of irradiation dose, but not in late harvest fruit. A similar study used 300 Gy doses and again showed no significant change in either AA or β-carotene levels after 4 and 6 days storage (Vanamala et al. 2005). A third study from the same group indicated significant losses of total vitamin C in two cultivars with very high doses of electron-beam irradiation (Girennavar et al. 2008). In this study, fruit were exposed to 1,000, 2,500, 5,000 and 10,000 Gy. These treatments are extreme but showed no significant change in Rio Red grapefruit with 1,000 Gy, but a statistically significant loss in Marsh White grapefruit at the same dose. However, at higher doses, large losses of vitamin C occurred in a dose-dependent manner, with losses of >50% at 10,000 Gy. In contrast, β-carotene levels were unaltered by irradiation in Rio Red grapefruit at any dose. But these iradition doses are ten times the levels used for quarantine treatment and are only presented as a guide for extreme high level treatment.

Lemon

Irradiation with 75 and 300 Gy had no significant effect on total vitamin C content in lemons up to three weeks after irradiation. Storage had little effect on vitamin C content in irradiated lemons (+1% and -5% change), while vitamin C content decreased 9% in non-irradiated lemons (Mitchell et al. 1992).

Lime

In limes, AA levels fluctuated during storage, but were decreased initially by doses of ≥500 Gy. AA levels remained lower in limes irradiated with ≥1,000 Gy during 90 days storage (Ladaniya et al. 2003).

Other non-vitamin bioactive compounds

Whilst citrus is a good source of traditional nutrients such as certain vitamins, minerals and fibre, nutritionists have more recently focused on a range of substances called 'non-vitamin bioactive compounds' or phytochemicals. An orange has over 170 different phytochemicals and more than 60 flavonoids which have been shown to have anti-inflammatory and anti-tumour activity as well as inhibiting blood clots and having strong antioxidant activity (Baghurst, 2000). This section reviews the use of low dose irradiation on these compounds.

Irradiation with 30, 54 and 164 Gy did not decrease total antioxidant capacity and total phenolic content in Clementine mandarins, and flavanone glycoside levels were similar or increased in irradiated fruit after 0 and 6 months storage (Contreras-Oliva et al. 2011). Irradiation of Clementines at a mean dose of 300 Gy followed by storage for 49 days at 3ºC resulted in enhanced synthesis of phenolic compounds, primarily hesperidin as the major flavanone glycoside, and nobiletin and heptamethoxyflavone as the major polymethoxylated flavones. Initially, the content of these flavonoids in peel was significantly lower than in controls but biosynthesis increased between days 14 and 21. The irradiation-enhanced content of these flavonoids and of para-coumaric acid, a biosynthetic precursor to the coumarins scopoletin and scopolin, may relate to enhanced resistance to mould decay, while the low irradiation dose and cold storage helped to minimize losses due to pitting of the peel (Oufedjikh et al. 2000). After 12 months storage, small decreases in flavanone glycoside levels occurred in fruit irradiated with 164 Gy (-7% to -12%). However, irradiation of clementine mandarins with 51 and 875 Gy did not alter flavanone glycoside levels after two months storage (Rojas-Argudo et al. 2012).

In grapefruit, effects of irradiation on flavanones were variable; higher doses (400 and 700 Gy) led initially to small reductions in naringin and narirutin in early season fruit, but these changes were lost after 35 days storage, and did not occur in late season fruit (Patil et al. 2004). However, total flavanone levels were increased by irradiation with 70 and 200 Gy after 35 days storage in early season fruit. Lycopene levels were similar between control and ≤1,000 Gy irradiated grapefruit, with the exception of a small decrease (~10%) after 35 days storage in late harvest fruit irradiated with 700 Gy (Patil et al. 2004; Vanamala et al. 2005; Girennavar et al. 2008). Lycopene levels were >25% lower in late compared to early harvest fruit, irrespective of irradiation. Limonin levels in grapefruit were also unaffected by irradiation with <1,000 Gy (Patil et al. 2004).

Table 5. Effects of irradiation on radiation-sensitive nutrients in citrus fruit.
Data primarily complied by FSANZ (2014) with addition of recent literature (2014- 2018).

Fruit	Dose (Gy)	Carotene	Vitamin C	Other components	Analysis method / Reference
Grapefruit	70, 200, 300, 400, 700	No change	No change	Flavonones: variable Lycopene: similar Limonin: no change	AA by HPLC Patil et al (2004) Vanamala 2005
Grapefruit (≥1,000 Gy)	1000, 2,500, 5,000, 10,000	No change	Dose-dependent decrease	Flavonoids and lycopene: No change with 1,000 Gy, variable effects with >1,000 Gy	Total vitamin C by HPLC Girennavar et al (2008)
Lemon	75, 300	n.d.	No change	n.d.	Total vitamin C by derivatization Mitchell et al (1992)
Lime Kagzi	0, 250, 500, 1,000, 1,500	n.d.	Variable. Decreased with 1,500 Gy[#]	n.d.	AA by titration Ladaniya et al (2003)[#]
Mandarin Clementine	30, 54, 164, 510, 875	n.d.	No change	Antioxidant capacity, phenolics: no change Flavanone glycosides: no change ≤6 months storage	Total vitamin C by HPLC Rojas-Argudo et al (2012) Contreras-Oliva et al (2011)
Mandarin Ellendale	75, 300	n.d.	No change	n.d.	Total vitamin C by derivatization Mitchell et al (1992)
Mandarin Imperial	75, 300	n.d.	-43%* and -60%* after 3 wk	n.d.	
Mandarin Nagpur	0, 250, 500, 1,000, 1,500	n.d.	Dose-dependent decreases for ≥ 500 Gy[#]	n.d.	AA by titration Ladaniya et al (2003)[#]
Orange Lane Late	200, 400 and 600	n.d.	No change	Phenolic content: no change Antioxidant capacity, phenolics: no change	AA by titration McDonald et al (2013)

Fruit	Dose (Gy)	Carotene	Vitamin C	Other components	Analysis method / Reference
Orange Kau	750	+33%* after 9 d	No change	n.d.	AA by titration Boylston et al (2002)
Orange Navel	200, 400, 600, 800 and 1,000	n.d.	Dependent on storage time	n.d.	Cho et al. (2015) Noh et al. (2016)
Orange Mosambi	0, 250, 500, 1,000, 1,500	n.d.	Immediate decrease with ≥1,000 Gy, but no difference after storage#	n.d.	Ladaniya et al (2003)#
Orange Valencia	10, 20, 30, 40, 50, 100, 150 and 200	n.d.	No change	n.d.	AA methods not reported De Bortoli et al (2015)
Orange blood	0, 250, 500	n.d.	AA higher in irradiated fruit after 1-6 weeks storage	n.d.	AA by titration Khalil et al (2009)

**Significant difference. n.d.; not determined.*
#AA determined, therefore some losses may be due to conversion to DHAA, and statistical analyses limit individual comparisons in this study.

In summary, Food Standards Australia New Zealand (FSANZ 2014) reviewed the published literature demonstrated that phytosanitary doses of irradiation on fresh fruit and vegetables and concluded that low dose irradiation:

- had no effect on carotene levels in fruits and vegetables
- did not decrease vitamin C levels in the majority of fruits and vegetables
- had little effect on other non-vitamin bioactive compounds.

In some cultivars of some fruits vitamin C levels decreased following irradiation. This was also seen in the citrus irradiation treatment literature (Table 5). However, in the majority of these cases the vitamin C content of irradiated fruit remained within the range of natural variation. In addition, when the effects of these changes were compared to dietary consumption patterns it was evident that these changes were unlikely to impact on dietary vitamin C intakes in Australia and New Zealand. FSANZ (2014) concluded that phytosanitary doses of irradiation do not pose a nutritional risk to the Australian and New Zealand populations and recommended that the data requirements for applications to irradiate fruits and vegetables can be streamlined to focus on data for vitamin C, with requirements for other nutrients to be determined on a case-by-case basis. In the case of citrus, the results above show that different citrus fruits and cultivars respond differently to irradiation treatments. This calls for the development of cultivar-specific irradiation treatment protocols. Vitamin C is water-soluble and it is sensitive to irradiative degradation, particularly at higher treatment doses (Kilcast 1994). To reduce vitamin C loss, irradiation treatments could be conducted at low temperatures (Mditshwa et al 2017).

Consumer acceptability

The consumer acceptability of irradiated fruit is the ultimate determinant of commercial acceptability. There have been limited studies on the trained panel and consumer acceptability of citrus following irradiation. Mc

Donald et al. (2013) examined the dose tolerance of Lane Late navel oranges to identify the sensory attributes that maybe affected by the treatment, and determine which changes, if any, influence consumer liking. They showed shows that trained panelists were able to detect increased pitting and visual damage in fruit treated by irradiation at 400 and 600 Gy and was corroborated by the consumer panels, which showed lower liking scores in overall appearance for the irradiated oranges compared to the control fruit. The effect was exacerbated by storage for 3 and 4 weeks (McDonald et al., 2013). Similarly, trained panelists in a study done by O'Mahony and Goldstein (1987) found increased brown blemishing and pitting of whole navel oranges irradiated at 300 and 600 Gy. However McDonald et al. (2013) found that overall liking was not affected by irradiation with similar overall liking scores for the control and treated fruit. Although consumers stated that they liked the appearance of untreated fruit significantly more than irradiated fruit, their overall liking of irradiated fruit was not different than control, although this could be a result of removal of the most heavily pitted fruit from consumer evaluation (McDonald et al., 2013). O'Mahony et al. (1985) also observed that untrained consumers were not able to tell the difference between untreated and irradiated navel oranges (600 – 800 Gy) even though expert judges were able to detect differences in brown blemishing and flavour of irradiated fruit after 5 – 6 weeks in storage. Betancurt et al. (2009) further showed irradiation (350 and 800 Gy) did not affect overall acceptability, appearance or juiciness of Late Valencia oranges after 20 and 40 days of storage as determined by 30 untrained panelists. While Hallman and Martinez (2001) found no differences detected by informal taste panels in Rio Red grapefruit, Marrs oranges, and Dancy tangerines treated up to 500 Gy and stored for 21 days at ambient temperature.

Noh et al. (2006a) evaluated the effects of 200, 400, 600, 800 and 1,000 Gy on Navel oranges and showed that after treatment with continuous storage at 20 ^{o}C for 12 days there was no differences in sensory acceptability of treated fruit. But after storage at 3 ^{o}C for up to 45 days, consumer perception of sweetness and overall acceptability of irradiated samples greater than 600Gy, were negatively affected by irradiation treatment (Noh et al. 2016b). McDonald et al. (2013) also showed there was a significant interaction between age and irradiation treatment suggesting that irradiation stresses the fruit and makes it more sensitive to handling. The Lane Late navel oranges used by McDonald et al. (2013) were commercially packed for the Pacific Rim (high export pack in which fruit are packed so as to place as much fruit as possible in the box). The fruit packs for visual damage evaluation were kept intact for the entire storage period. For other evaluations, fruit was removed from the original packs; this latter sample did not show as much visual damage as the fruit that was tightly packed. In addition, the fruit used in this study were late season navels, which had suffered stress due to a wet winter. This may also have contributed to the greater impact of irradiation. This observation suggests that packing compression in conjunction with the irradiation may enhance bruising of navel oranges. O'Mahony and Goldstein (1987) also observed greater pitting in navel oranges of damp oranges. The effect of handling, packing density as well as time of harvest should be evaluated in future studies.

While fruit softening has been shown to be not affected to irradiation in Late Valencia fruit (Betancurt et al., 2009), it has also been measured to increase (Miller et al., 2000; O'Mahony et al., 1985; McDonald et al., 2013) but this increased softness has not been able to be detected by trained panelists (O'Mahony et al., 1985; McDonald et al., 2013). Although O'Mahony et al. (1985) rated the resistance to bite of the treated oranges to be slightly higher compared to the control. Similarly McDonald et al. (2013) showed that trained sensory panelists were unable to determine differences in internal dryness or granulation among the control and irradiation treatments, even though analytical testing had indicated that there was a positive association of irradiation with segment drying at 400 and 600 Gy and with granulation at 600 Gy. This difference in results could have been due to the fact that the trained panelists were evaluating fruit sections rather than whole slices or that the drying and granulation, although present in a number of the fruit, was fairly minor on a whole fruit basis. The low prevalence of the drying and granulation was also indicated by results from the consumer panels, which found there to be no differences among treatments for either texture or juiciness.

McDonald et al. (2013) also found that the 400 and 600 Gy doses of irradiation enhanced the concentrations of aroma volatiles present in the Lanes Late but again this did not appear to result in a loss in flavor quality given that both the trained and consumer sensory panels which found there to be no significant differences in the flavor or overall liking between the control and irradiated fruit. The lack of sensory impact of the increases in volatile concentrations was also noted in sensory panel evaluations of aroma of both whole and cut fruit, which found no differences due to treatment (McDonald et al, 2013). Although there was no sensory impact of the increases in aroma volatiles, these changes indicate that irradiation is inducing metabolic alterations in the fruit that have the potential to alter flavor at higher dose levels (McDonald et al., 2013). Similarly McDonald et al. (2013) showed there was decreased TSS at doses 400 and 600 Gy, these small effects had little impact on flavour. Indeed with other citrus types such as pummelos, while Jain et al. (2017) showed no consistent effects of 150 and 1,000 Gy irradiation on TSS and TA values, these differences were low and did not affect consumer acceptability of the treatments (Jain et al., 2017).

In summary, McDonald et al. (2013) concluded that while there maybe some measurable effects of irradiation on fruit quality (such as aroma volatiles, internal drying/granulation, TA, and TSS) these have not been shown to influence perceived flavour as indicated by the trained sensory panel or consumers.

Observations and opportunities

From a postharvest perspective, the development of physical damage to the peel following treatment is a consequence of localized uncontrolled senescence and necrosis. However there have been very few studies to investigate different pre- and postharvest treatments that could minimise or prevent this damage. All previous irradiation research has been simple empirical research, where the experiment has generally examined different irradiation doses on a single batch of fruit. This type of research is beneficial to compare different irradiation doses, but does not give any information on how irradiation effects quality and how the observed differences can be better managed.

Dr. Marisa Wall from ARS / USDA concluded from her extensive experience and review of irradiation effects on fruit quality that cultivar effects may be related to slight variations in maturity at harvest, and few carefully designed studies have differentiated these factors (Wall, 2015). Hence the differences observed between the different cultivars maybe due to different maturities when treated. While this somewhat problematic for citrus, Jessup et al. (1992) showed green Lisbon lemons were more severely affected by low dose irradiation than yellow lemons. Therefore objective assessments of physiological maturity are needed to evaluate multiple cultivars for sensitivity to irradiation during quarantine treatment. Furthermore there could be other pre- and postharvest factors that could affect the fruit response to irradiation. In other areas of postharvest storage and treatment of citrus, the use of hot fungicides, treatment temperatures and the use of curing have been shown to reduce physical damage to citrus peel following postharvest stresses (such as chilling). These factors have not been assessed in their effects on irradiation damage and are worth exploring to minimise any potential damage.

Conclusions

Low dose irradiation is an important market access tool which can be used for the export of Australian citrus to some markets. This review showed that in general there are few consistent negative effects of low dose irradiation treatment and subsequent cold storage on citrus quality. While some minor quality issues with internal quality were identified, these are generally not consistent and even if these differences are statistically significant they are often not commercially significant. However there are some commercial issues identified with the development of disorders or damage (such as pitting, browning of the calyx and loss of firmness) due to treatment in some types and cultivars of citrus. This damage can be commercially unacceptable and may limit the use of irradiation for some citrus types and cultivars. However this damage is cultivar and even harvest-time specific and more work is required to identify these hybrids / cultivars in local conditions. Better understanding the causes of potential damage offers the potential of relatively simple harvest and postharvest treatments to better manage any potential treatment effects.

Recommendations

Fruit quality issues such as the development of chilling injury during the current cold treatment protocol for chilling sensitive citrus types (particularly lemons) can potentially be overcome with the use of low dose irradiation. However the commercial acceptance of low dose irradiation as a market access treatment will rely on its routine efficacy to maintain fruit quality through the supply chain. While this review identified some fruit damage in response to low dose irradiation, more work is required to identify and quantify the conditions and citrus types where this damage does not occur.

It is recommended to focus research on citrus types where the current cold treatment can cause chilling injury and limits out-turn on export markets. Lemons and to a lesser extent mandarins should be a focus which could benefit from future research and development investments to improve quality out-turn. Many previous studies have only evaluated a single type and cultivar of citrus harvested from a one geographic area location at one time period. This work should be expanded to include multiple varieties harvested at different times (even years), and from varied locations. This will give growers, exporters, importers and the market confidence in the commercial use of this treatment. Following on from the work of McDonald et al. (2013) who showed some significant skin damage following irradiation in five orange cultivars and five mandarin cultivars, further research should also consider pack configuration on sensitivity of fruit to damage. In addition, it is recommended to explore the pre-treatment effects such as harvest time, postharvest storage and treatment (e.g. hot fungicides and curing), on the fruit response to irradiation. It may be possible to minimise the potential negative effects of irradiation with simple curing or management issues before treatment.

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