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Effect of Phytosanitary Irradiation Treatment on the Storage Life of 'Jiro' Persimmons at 15 °C

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Abstract: Irradiation is becoming a more accepted phytosanitary market access treatment for some international horticultural trades. However, there is little information on the effects of phytosanitary irradiation treatment on persimmon fruit quality. 'Jiro' persimmon fruit were treated with an average of 769 Gray (Gy) at a commercial phytosanitary irradiation X-ray facility to examine the effect of this market access treatment on fruit quality during storage. After treatment, fruit were stored in air at 15 °C for up to three weeks. The results showed that, in general, there was no effect of irradiation treatment on fruit weight loss, calyx appearance, fruit firmness (objective and subjective), total soluble solids (TSS), titratable acidity (TA), internal appearance, and ethylene production rate. There were some treatment differences in fruit respiration rates and some aspects of fruit appearance and colour, where irradiated fruit had higher respiration rates and were slightly darker with higher levels of skin blemish, although these measured differences were not commercially significant. This study showed the promise of using low dose irradiation as a phytosanitary treatment for 'Jiro' persimmons, but more work is required to test other persimmon cultivars and other storage and marketing environments.

Keywords: persimmon; irradiation; quality; storage; market access

1. Introduction

Persimmons (*Diospyros kaki* L. f.) are a popular fruit, with world production being at around 8 million tonnes per year [1]. World trade in persimmon fruit is increasing, but persimmon fruit are perishable and are also hosts to quarantine pests such as Mediterranean fruit fly (*Ceratitis capitata*), which require a postharvest disinfestation treatment to ensure pest freedom if the fruit is to be traded into sensitive export markets. While established phytosanitary treatments such as methyl bromide fumigation and cold treatment have been used as end-point treatments to ensure pest freedom, each of these treatments have their limitations such as the use of postharvest chemical fumigants or long cold treatment times [2]. In addition, alternative phytosanitary treatments such as controlled atmospheres and radiofrequency heating have been studied for their effects on fruit quality [3,4].

The development of irradiation as an alternative disinfestation technology has been sporadic, mainly because of access to alternative treatments, cost of treatment, and the imposition of long-term moratoria in some countries. However, irradiation is a technologically proven, viable, and scientifically sound disinfestation treatment [5]. Moreover, irradiation is increasingly becoming an approved and agreed treatment in world trade of food and horticultural products [2]. The trade of irradiated produce is based on the International Standards for Phytosanitary Measures (ISPM), 'Phytosanitary Treatments

for Regulated Pests' (ISPM 28), which regulates the minimum doses for a range of regulated pests [6]. This landmark development for the use of irradiation as phytosanitary treatment recognises that a minimum 150 Gy treatment is a generic dose that will prevent the emergence of all adult Tephritid fruit flies in any host commodity.

The availability of generic radiation phytosanitary treatments, such as ISPM 28, has stimulated worldwide interest in irradiation as a market access treatment [7,8]. While the ISPMs which allow irradiation as a market access treatment are signed under the International Plant Protection Convention, the acceptance of irradiation as a treatment is left to the discretion of each country. While approximately 60 countries have approved the use of food irradiation in their health or food regulations for at least one food (e.g., dried spices), its acceptance for fresh fruit is more limited, where over 40 countries allow the trade of irradiated fresh fruit and vegetables [2].

There have been very few studies on the effects of irradiation as a quarantine treatment on persimmon fruit with low dose phytosanitary treatment doses [9,10], with other studies conducted on higher irradiation doses above the phytosanitary limit (1000 Gy maximum). For example, 'Hachiya' persimmons were treated with a range of irradiation doses (1500–3500 Gy) [11], but these treatment doses are not representative of any commercial market access treatment [6]. 'Fuyu' persimmons have been treated with 300 and 1000 Gy in combination with modified atmosphere (MA) bags and showed some skin blemish on 1000 Gy treated fruit stored in MA bags after removal from cold storage [9].

Thailand currently allows access for the import of persimmons from some areas of Australia (Queensland) with irradiation treatment as a phytosanitary treatment at either 150 Gy for Tephritid fruit flies (such as Queensland fruit fly (*Bactrocera tryoni*) or at a minimum of 400 Gy for other insect pests except pupae and adults of the order Lepidoptera [12]. This treatment has allowed successful trade of persimmons from Queensland, but growers in other Australian production areas would like to access this export market using irradiation as a phytosanitary treatment. This preliminary study examined the effect of commercial phytosanitary low dose irradiation (0 or 550 Gy) treatment on the storage and shelf life of 'Jiro' persimmons from New South Wales, Australia, following 15 °C storage for up to three weeks. While 15 °C is not the ideal storage temperature for persimmons, this temperature was chosen as a representative of an export supply chain. The single commercial irradiation treatment dose was selected as a previous study showed no differences in irradiation dose (200, 400, 600, and 800 Gy) on 'Fuyu' fruit quality following treatment and storage [10]. 'Jiro' along with 'Fuyu' belong to the pollination constant non-astringent group of persimmons, which are non-astringent fruit.

2. Materials and Methods

2.1. Plant Materials and Experimental Procedure

'Jiro' persimmon fruit were harvested from a commercial orchard in Barooga, New South Wales in Australia (35°54′0″ S, 145°41′0″ E) on 3 May 2020 at commercial maturity (i.e., full orange colour with no visible green, at least 15% TSS and firm flesh) [13]. After harvest, fruit were transported overnight to Melbourne (265 km) in a refrigerated truck to the commercial X-ray irradiation facility (Steritech Pty Ltd. in Merrifield, Melbourne, Australia) for irradiation treatment. Treated fruit were exposed to X-rays from a source with a beam energy of 5 MeV to reach a dose target dose of 550 Gy. This was applied to 6 trays of fruit. Actual doses were determined by placing alanine dosimeters (Harwell Dosimeters, Oxfordshire, UK) within each tray. The actual average delivered dose was 769 Gy (dose uniformity ratio = 1.19). Untreated control (UTC) fruit were similarly handled but not treated with irradiation (0 Gy).

Following treatment, fruit were transported by refrigerated truck to Centre of Excellence for Horticultural Market Access at NSW Department of Primary Industries at Ourimbah (950 km) where fruit were stored at 15 °C for up to three weeks. Transport temperatures were monitored with calibrated temperature loggers (TinyTag TV-4020 loggers, Chichester, Gemini Data Loggers, UK) and showed fruit temperatures during transport were between 12–15 °C. The experiment was designed as a split plot

with three replicates. The two irradiation treatments (0, target 550 Gy) were assigned to whole plots (total of 6), while the two assessments were assigned to sub-plots. Each experimental unit consisted of a tray of 20 fruit. After treatment, fruit were non-destructively assessed at 3, 7, 14, and 21 days. Fruit were destructively assessed after 1 and 3 weeks of storage at 15 °C and all assessments were conducted on fruit that had warmed to 20 °C.

2.2. Physio-Chemical Assessments

Fruit quality was assessed by measuring fruit weight loss, skin colour, skin blemish, calyx appearance, fruit firmness (objective and subjective), total soluble solids (TSS), titratable acidity (TA), internal appearance, respiration rate, and ethylene production rates.

2.2.1. Weight Loss

Weight loss of the fruit was assessed using an electronic balance (Model Kern & Sohn, Balingen, Germany), where each individual fruit were labelled and fruit weight was recorded at the beginning of the experiment and at each subsequent assessment day. Weight change was expressed as a percentage value determined by deducting the initial weights (W1) from the final weights (W2) divided by the initial weights and multiplied by 100 percent (%).

2.2.2. Skin Colour and Blemish

Skin colour was quantified on the equatorial region on opposite sides of each fruit using a Minolta Chroma Meter model CR-400 (Minolta Co. Ltd., Osaka, Japan). Each individual fruit per replicate was assessed (20 fruit per replicate). Colour was measured using the 'Lab' colour space system [14]. The levels of blemish on the skin were also visually rated as % of the fruit surface affected by blemish based on the following scale: 0 = no blemish (0% blemish); 1 = trace (<10% blemish); 2 = slight (11–25% blemish); 3 = moderate (26–50% blemish), and 4 = severe (>51% blemish).

2.2.3. Calyx Condition

The general appearance of the calyx on each individual fruit (20 fruit per replicate) was visually rated using the following rating scale: 0 = fully green, fresh; 1 = slight browning; 2 = moderate browning/drying, 3 = severe browning/drying, and 4 = completely brown/dried.

2.2.4. Flesh Firmness

Subjective fruit firmness was assessed by gently squeezing each individual fruit (20 fruit per replicate) and using the following scale: 0 = hard; 1 = rubbery; 2 = sprung; 3 = firm soft, and 4 = soft. Objective flesh firmness was determined with a hand-held penetrometer (Effegi hand-held firmness tester, Facchini, Alfonsine, Italy) mounted on a drill press. Flesh firmness was measured in the flesh with the peel removed at two locations at the equatorial region on opposite sides of each fruit.

2.2.5. Respiration Rate and Ethylene Production Rate

Fruit respiration and ethylene production rates were determined on fruit that were sealed into an airtight 1.7 L glass jars fitted with a septum for different incubation times to accumulate respiratory gases. Two fruit were sealed in each jar, and two jars of fruit were used in duplicates for each replicate. Ethylene concentration in the jar was determined by withdrawing a 1 mL gas sample from the headspace and injecting into a gas chromatograph (Gow-Mac Model 580, Bridgewater, NJ, USA) fitted with an alumina column and flame ionisation detector. Operating temperatures for the detector, injector, and column were 110 °C, 50 °C, and 110 °C, respectively. The carrier gas used was high purity nitrogen (BOC Gases, Sydney, NSW, Australia) at a flow rate of 25 mL min^{-1} [15]. Fruit respiration rates were measured as carbon dioxide (CO_2) production (mL CO_2 kg^{-1} h^{-1}), and ethylene production rate was measured as μL C_2H_4 kg^{-1} h^{-1}.

2.2.6. Internal Fruit Quality Assessments

The internal appearance of each individual fruit (20 fruit per replicate) was assessed after cutting the fruit equatorially and assessed for gelling appearance using a five-point subjective scale: 0 = no gelling symptoms; 1 = start of some initial symptoms; 2 = slight gel symptoms; 3 = moderate gelling and 4 = severe gelling symptoms.

Total soluble solids (TSS, % Brix) was measured using a digital refractometer (Atago, Tokyo, Japan) on the juice collected from 10 fruit per treatment and expressed as % Brix. Titratable acidity (TA) was measured on a combined juice sample of 10 fruit per treatment. TA was determined by titrating 5 mL of the juice with 0.1 M NaOH to pH 8.2 with an automatic titrator (Mettler Toledo Titration Excellence T50 Titrator with a DG101-SC electrode, Switzerland) and results expressed as percentage citric acid [16].

2.3. Statistical Analysis

Although the irradiation dose was separately measured in each box during treatment, the irradiation treatment was not repeated. The differences between the treatment and the untreated control samples may be confounded with other sources of error. In the statistical analysis of the data, it is assumed that the differences in fruit responses are due solely to the differences in irradiation treatment where data were subjected to one-way analysis of variance (ANOVA) and the least significance difference (LSD) test using the SAS statistical software version 9.4. Data are reported as means and differences between the means were considered statistically significantly different at $p < 0.05$.

3. Results and Discussion

3.1. Changes in External Quality

There were few differences in the fruit skin colour of 'Jiro' persimmons (as measured with the Minolta colour meter) within the first week of storage at 15 °C (Figure 1). However, after 7 days of storage at 15 °C, treated fruit tended to be darker orange/red, as noted with lower "b" value, hue angle and L* values in treated fruit (Figure 1). This is also illustrated in Figures 2 and 3, which show the photos of the fruit three days after treatment (Figure 2) and after three weeks storage at 15 °C (Figure 3), where these observed differences between the treatments were not outside commercial acceptability. Previous research on the effects of low dose irradiation on persimmon fruit quality have shown no effect of irradiation treatment on 'Fuyu' fruit colour [9,10].

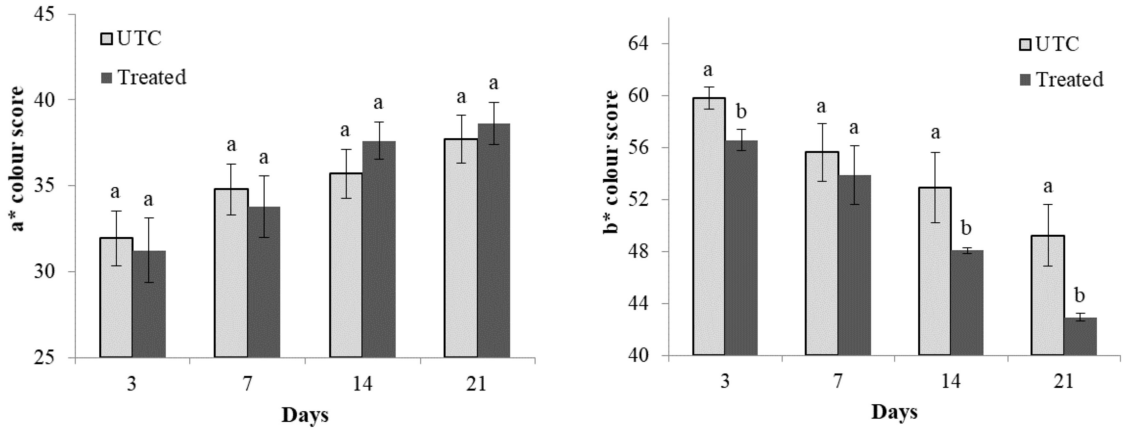

Figure 1. *Cont.*

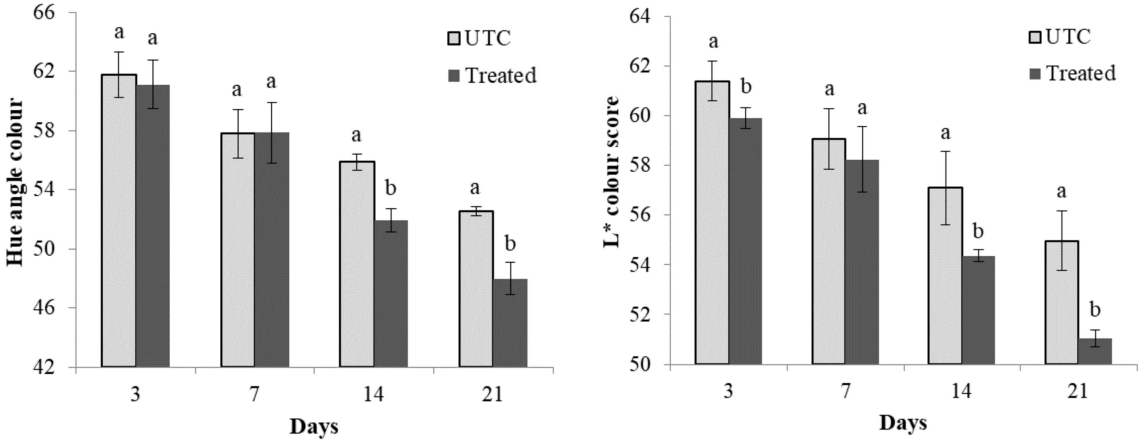

Figure 1. Skin colour parameters (a*, b*, hue angle and L*) of treated and untreated control (UTC) 'Jiro' persimmon fruit during storage at 15 °C for up to three weeks. Data are means (n = 3) and standard deviations around the mean are shown in error bars. Values with the same letter within each assessment time are not significantly different using the LSD test at $p < 0.05$.

Figure 2. *Cont.*

Figure 2. Untreated control (UTC) (**top**) and treated (**lower**) 'Jiro' persimmon fruit three days after treatment.

Figure 3. Untreated control (UTC) (**top**) and treated (**lower**) 'Jiro' persimmon fruit after three weeks storage at 15 °C.

There was no effect of irradiation treatment on fruit weight loss during storage at 15 °C (Table 1). This is in contrast to Wheeler et al. [9], who showed higher water loss in 1000 Gy treated 'Fuyu' treated persimmon fruit. As expected, fruit weight loss increased during storage. However, there were no differences detected between the treatments. Weight loss is often equated to water loss, and water loss is thought to contribute to the loss of calyx condition in persimmon fruit [17]. In this experiment, there was progressive drying out (browning) of the calyx during storage but there were no discernible differences between the treated and non-treated fruit (Table 1). Water loss is not only important in the cosmetic appearance of the calyx, Nakano et al. [18] showed that the rapid fruit softening in 'Tonewase' persimmon fruit can be caused by the action of ethylene that the calyx produces in direct response to water stress. In this experiment, there were no differences were detected between the treatments in relation to fruit ethylene production rates (Table 1).

Table 1. Effect of irradiation treatment on 'Jiro' persimmon fruit quality during storage at 15 °C for up to three weeks. Treatment comparisons are made within each assessment time.

	Assessment Time							
	3 Days		7 Days		14 Days		21 Days	
Fruit Quality Parameter	**UTC**	**Treated**	**UTC**	**Treated**	**UTC**	**Treated**	**UTC**	**Treated**
Weight loss (%)	-	-	1.8^{a}	1.9^{a}	-	-	3.3^{a}	3.4^{a}
Calyx freshness score	2.3^{a}	2.3^{a}	1.9^{a}	2.0^{a}	2.5^{a}	2.8^{a}	3.4^{a}	3.6^{a}
Flesh firmness score	0.5^{a}	0.6^{a}	0.9^{a}	0.9^{a}	1.5^{a}	1.5^{a}	1.6^{a}	1.5^{a}
Flesh firmness-penetrometer (kg firmness)	-	-	5.4^{a}	4.9^{b}	-	-	2.1^{a}	2.3^{a}
Total soluble solids (TSS, % Brix)	-	-	17.6^{a}	17.7^{a}	-	-	17.8^{a}	17.5^{a}
Titratable acidity (TA, % citric acid)	-	-	0.088^{a}	0.079^{a}	-	-	0.057^{a}	0.071^{a}
Internal gelling score	-	-	2.0^{a}	2.0^{a}	-	-	2.5^{a}	2.6^{a}
Ethylene production rate ($\mu L\ C_2H_4\ kg^{-1}\ h^{-1}$)	n.d.	n.d.	n.d.	n.d.	0.062^{a}	0.103^{a}	0.036^{a}	0.106^{a}

"-" denotes not measured at that assessment time. Calyx freshness score: 0 = fully green, fresh; 1 = slight browning, still good; 2 = moderate browning/drying, 3 = severe browning/drying, and 4 = completely brown/dried. Flesh firmness score: 0 = hard; 1 = rubbery; 2 = sprung; 3 = firm soft, and 4 = soft. Internal gelling score: 0 = no gelling symptoms; 1 = start of some initial symptoms; 2 = slight gel symptoms; 3 = moderate gelling and 4 = severe gelling symptoms. Different letters indicate mean values in the same rows and storage time are statistically different using the LSD test at $p < 0.05$. n.d. = not detected (limit of detection <0.001 $\mu L \cdot L^{-1}$ ethylene).

The effects of irradiation treatment on fruit respiration rates are presented in Figure 4 and show that the treated persimmon fruit had higher respiration rates than untreated fruit. Similar results have been observed where irradiated 'Fuyu' persimmon fruit had higher respiration rates during storage [9,10]. Higher fruit respiration rates during storage can indicate fruit stress or more mature fruit [19] and can be exacerbated with the use of MA bags, which are common for the long-term storage of persimmon fruit.

Skin blemish is a negative consumer attributes and many postharvest phytosanitary treatments can induce skin browning in persimmons [3]. In this experiment, irradiation treatment had a negative effect on the level of superficial skin blemish on the fruit surface during storage from 7 days of storage (Figure 5). A similar result was observed with 'Fuyu' fruit treated with 1000 Gy and stored in MA bags for two weeks [9]. However, in this experiment, while the differences in skin blemish detected in this experiment were statistically significant, these levels of blemish were assessed as 'minor' levels (i.e., with <10% blemish). The levels of blemish can also be compared in the photos of fruit in Figure 3. Na et al. [20] showed that 'Jiro' persimmon fruit are less susceptible to fruit browning at the calyx-end

than other cultivars such as 'Fuyu', 'Uenishiwase', 'Daiandangam', and 'Ro-19' and therefore a broader scope of persimmon cultivars for testing with irradiation treatment is required.

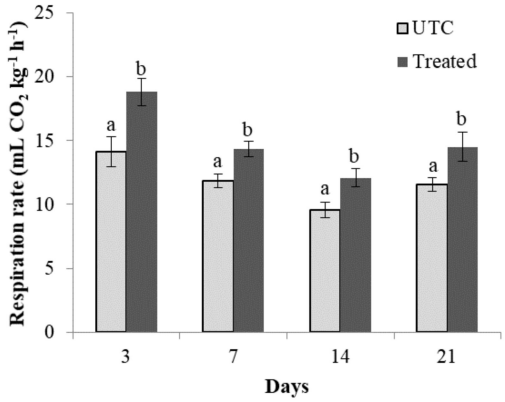

Figure 4. Fruit respiration rate (mL CO_2 kg^{-1} h^{-1}) of treated and untreated (UTC) 'Jiro' persimmon fruit during storage at 15 °C for up to three weeks. Data are means (n = 3) and standard deviations around the mean are shown in error bars. Values with the same letter within each assessment time are not significantly different using the LSD test at $p < 0.05$.

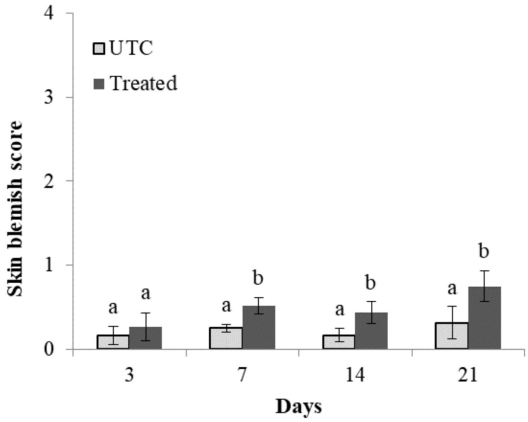

Figure 5. Skin blemish of treated and not treated (UTC) 'Jiro' persimmon fruit during storage at 15 °C for up to three weeks. The skin was visually rated as % of the fruit surface affected by blemish based on the following scale: 0 = no blemish (0% blemish); 1 = trace (<10% blemish); 2 = slight (11—25% blemish); 3 = moderate (26–50% blemish), and 4 = severe (>51% blemish). Data are means (n = 3) and standard deviations around the mean are shown in error bars. Values with the same letter within each assessment time are not significantly different using the LSD test at $p < 0.05$.

Fruit firmness is an important consumer trait of persimmons [17]. In this experiment, flesh firmness was assessed in two ways; subjectively evaluated by gently squeezing the fruit, and objectively by measuring firmness using a penetrometer. The results presented in Table 1 show the fruit became progressively softer during storage, particularly after 7 days of storage. The results of the destructive objective measurement of firmness using a penetrometer showed there was no difference in flesh firmness between the treatments. This was supported with the observations of the subjective fruit firmness, where no differences in the subjective physical feel of the fruit were detected between the treatments. However, other studies have shown irradiation treatment at 400 and 800 Gy resulted in softer 'Fuyu' fruit after 60 days of storage at 0 °C [10]. These observed differences in fruit firmness responses to irradiation treatment may be due to the differences in storage temperature and time.

3.2. Changes in Internal Quality

The effect of irradiation treatment on TSS content of 'Jiro' persimmon fruit is presented in Table 1, and the results show there was no effect of treatment on the levels of TSS during storage. Similar observations have been observed in other studies with 'Fuyu' fruit [9]. Persimmons do not have high levels of TA in the fruit, and no differences in TA levels between the treatments during storage were detected.

Internal gelling is a characteristic of fruit senescence [17]. In this experiment, the level of gelling increased with storage time, but there were no differences detected between the treatments (Table 1). Cross-sections of the treated and untreated fruit that had been cut in half after three weeks of storage are presented in Figure 6 and illustrate the level of internal gelling of the fruit after storage.

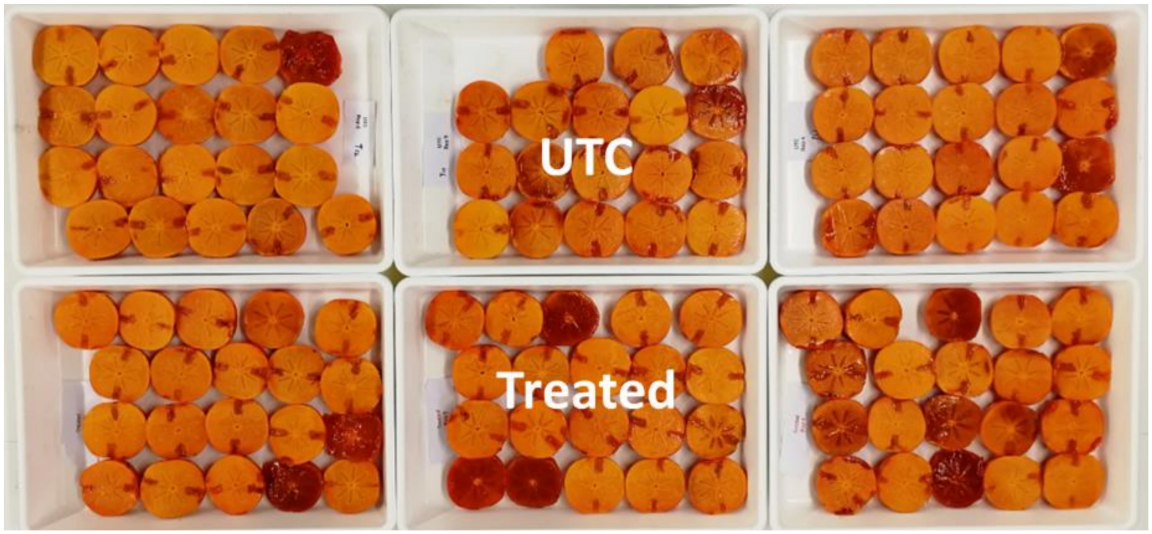

Figure 6. Untreated control (**top**) and treated (**lower**) 'Jiro' persimmon fruit cut in half (after firmness measurement = hole/darkened in the side of the fruit) after three weeks storage at 15 °C. Darker fruit are fruit with full internal gelling symptoms.

4. Conclusions

This experiment examined the effect of storage time and phytosanitary irradiation treatment on the quality of 'Jiro' persimmon fruit during storage at 15 °C. In general, there was no effect of irradiation treatment on fruit quality. As expected, storage time had an effect on fruit quality over time with the fruit becoming softer, losing weight, and drying of the calyx appearance (i.e., browning) with longer storage times. Respiration rates were higher in treated fruit and indicated some fruit stress caused by the treatment, but this did not translate into lower fruit quality. Indeed, there was no effect of irradiation (i.e., no difference when compared to non-irradiated control fruit) on fruit weight loss, calyx appearance, fruit firmness (objective and subjective), TSS, TA, internal appearance, and ethylene production rate. While there were some differences in fruit colour and skin blemish, where irradiated fruit were darker and had higher levels of skin blemish at the end of the storage, these differences were not commercially significant. This is a preliminary experiment and scoping study that was limited due to the limited number of irradiation treatment doses, persimmon cultivars, storage treatments etc., but it did, for the first time, report on the effect of irradiation on 'Jiro' fruit quality following storage. Further extension to other irradiation treatment doses and persimmon cultivars and storage conditions are needed to confirm these findings on a broader range of persimmon fruit and marketing conditions.

Author Contributions: J.B.G. conceived the research hypothesis that led to the experiment, contributed to the experimental design, carried out the experiment and wrote the manuscript draft, B.W. contributed to the experimental design, carried out the experiment, contributed to data analysis and edited the manuscript draft, P.P. contributed to the experimental design, analysed the data and edited the manuscript draft. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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