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Utilisation of hot water treatment on papaya (*Carica papaya* L. cv. Eksotika II) to elucidate disease resistance and maintain postharvest quality

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Abstract

Papaya fruit (*Carica papaya* L.) is one of the most widely farmed fruits in Malaysia, and produced for domestic consumption and exported worldwide. Papaya fruit is susceptible to anthracnose, a fungal infection caused by *Colletotrichum gloeosporioides* that negatively affects fruit quality and shelf-life. The common disease control approach utilises fungicides such as prochloraz as a postharvest application. However, public concerns regarding the health risks of fungicide residue on food have created interest in safer and greener alternatives. As a result, hot water treatment at 54°C for 5 min was investigated in order to reduce or replace the reliance on fungicides. Results showed that papaya fruits treated with hot water presented a higher reduction in disease incidence and severity. Additionally, hot water treatment preserved the physicochemical properties, prolonged shelf-life, and increased the papaya fruits' total phenolic and flavonoid contents while up-regulating metabolites that are involved in stress tolerance.

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Introduction

Papaya (*Carica papaya* L.) is a major fruit grown in Malaysia for domestic consumption and exported worldwide. This makes Malaysia as one of the world's major players in papaya export, with revenue of RM 100 to 120 million per year (FAOSTAT, 2019).

However, about a third of the fruits produced are lost postharvest due to diseases and rots. Among the most economically significant postharvest diseases in papayas is anthracnose caused by *Colletotrichum gloeosporioides* (Ayón-Reyna *et al.*, 2017a). The hot and humid climate of Malaysia is highly conducive for the fungus, which results in year-round infections. The fungus could infect the young fruits on the field, remain latent, and only show symptoms once the fruits ripen. These lesions rapidly enlarge, and could cover the whole fruits as they continue to ripen, thus rendering them inedible (Sarkar *et al.*, 2016). This is compounded by another problem caused by the fruit's limited shelf-life (Gao *et al.*, 2020), which also leads to substandard appearance, texture, flavour, and overall quality, and can account for up to 25% of postharvest losses. Furthermore, the rigorous selection of fruits for exports leads to rejections of up to 30%. As a result, significant fraction of fruits is discarded (Mishra *et al.*, 2015).

The common disease control approach utilises fungicides such as prochloraz or propiconazole as a postharvest application. However, public concerns regarding the health risks of fungicide residue on foods, and the potential development of resistant crops have created interest in safer and greener alternatives (Ayón-Reyna *et al.*, 2017a).

Physical postharvest heat treatments (water or vapour) are considered to be non-polluting and safe. In the fruit industry, disease management and insect disinfestation control are crucial for postharvest storage and marketing. The treatment is effective in controlling several fungal pathogens such as *Botrytis cinerea* (Di Francesco *et al.*, 2018), *Colletotrichum musae* (Vilaplana *et al.*, 2018), and *Gloeosporium* spp. (Prunier *et al.*, 2018). Heat treatments at 54°C for 4 min altered the quality of papaya fruits, whether for the control of chilling injuries, diseases, or insects. However, the heat treatment of papaya fruits does not

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change the internal colour, total soluble solids, weight losses, and concentrations of β-carotene and lycopene (Terao *et al.,* 2019).

Antioxidant activity is a term that refers to a bioactive substance's ability to maintain cell structure and function, effectively neutralise free radicals, suppress lipid peroxidation, and protect cells from future oxidative stress (Bravo, 1998). Several studies have reported that the application of heat increases antioxidant enzymes and phytochemicals such as carotenoids and phenolic compounds. Ummarat *et al.* (2011) reported that bananas treated with hot water exhibited an increase in antioxidants, thus indicating an induction of antioxidants. Talcott *et al.* (2005) discovered an increase in polyphenols and carotenoids in hot water-treated mangoes, thus resulting in increased antioxidant activity as compared to untreated mangoes. These investigations, however, have not proven a correlation between treatment with hot water (54°C at 5 min) followed by storage at room temperature (25°C), and the antioxidant chemical content and fruit quality of papaya fruits.

Therefore, the present work investigated the efficacy of hot water treatment (HWT) in controlling anthracnose disease in papaya fruits. The major aims of the present work were to examine *C. gloeosporioides* incidence and severity on hot water treated papaya fruits, and to analyse several physicochemical attributes and antioxidant properties of hot water treated papaya fruits during postharvest storage. The present work also aimed to identify metabolites that are present in the treated papaya fruits.

Materials and methods

Plant material

A local exporting company provided mature green papaya fruits (*Carica papaya* L. cv. Eksotika II) which were green with shades of yellow on the skin; colour index 2 (Lam and Zaipun, 1987), and harvested on the same day. Fruits of consistent size (450 - 550 g), shape, development, maturity, as well as the absence of any signs of mechanical damage, insect infestation, or infection were selected for experimental purposes.

Inoculum preparation

Papaya fruits were placed in plastic basins holding wet paper towels, sealed with Parafilm, and left at room temperature (25°C) for 24 h. Under an optical microscope, papaya fruits with common anthracnose signs or rot lesions were studied. Small fragments of tissue were dissected from the edge of a lesion that was actively growing, and surface-sterilised for 2 - 3 min in 1.0% sodium hypochlorite (NaOCl) to sterilise the surface of the fruits from debris, and to remove fungal inoculum found on the surface of the fruits, followed by washing with sterile distilled water. Then, the fruit tissues were dried on sterile filter paper, plated on Potato Dextrose Agar (PDA; Difco, USA), and incubated at 25 ± 2°C for 8 d. After observing mycelial growth, colonies were re-isolated on fresh PDA plates to obtain pure cultures. Each Petri dish was filled with 10 mL of sterile water and flocculated. *C. gloeosporioides* was isolated and morphologically characterised from anthracnose infections (Damm *et al.,* 2012). The conidial suspension was filtered twice *via* gauze, and the concentration was determined using a Neubauer haemocytometer, and corrected to 10^6 conidia/mL (Batista *et al.,* 2014). *C. gloeosporioides* cultures were maintained on PDA agar, and sub-cultured every week.

Hot water treatment (HWT)

Randomly selected fruits were rinsed and immersed for 10 min in 0.3% hypochlorite solution. The fruits were then cleaned with running tap water, and air-dried at 25°C. Preliminary research indicated that treating papaya fruits with 54°C for 5 min was the most efficient method of managing infections and delaying fruit ripening. Whole papaya fruits were submerged in hot water (54°C) for 5 min, removed, and left to dry naturally at 25°C. The control group (untreated) was immersed in room temperature (25°C) water for 5 min. After air-drying, the control group and a portion of the HWT group were inoculated with the conidial suspension (HWT-CG). The other portion of the HWT group was inoculated with distilled water (HWT-DW). A total of three fruits were used for each treatment, and all experiments were repeated three times. For each HWT treatment and control, nine biological replicates (n = 9) were employed. After treatment, papaya samples were placed onto rectangular polypropylene basins, sealed with stainless steel wire (0 - 10 squares per inch), and stored at 25°C for 8 d. All analysis was conducted in triplicates. Table 1 lists the treatments performed on the papaya samples.

Table 1. Papaya treatments.

Sample	Treatment	
Control	Control (Inoculated with *C. gloeosporioides)*	Dipped in distilled water for 5 min
HWT	HWT-CG (Inoculated with *C. gloeosporioides)*	Dipped in hot water (54°C) for 5 min
	HWT-DW (Inoculated with distilled water)	Dipped in hot water (54°C) for 5 min

HWT: hot water treatment; HWT-CG: hot water treated fruits inoculated with *C. gloeosporioides*; and HWT-DW: hot water treated fruits inoculated with distilled water.

Disease incidence and lesion diameter

Disease incidence (DI) was recorded in accordance with the symptoms of anthracnose on fruit surfaces (Sivakumar *et al.*, 2002a). On 0, 1, 2, 4, 6, and 8 days under ambient storage at room temperature, the effect of hot water treatment on DI was examined. The DI (%) was expressed as the number of fruits showing anthracnose out of the overall number of fruits in each treatment using Eq. 1:

$$Disease\ incidence\ (\%) = \frac{Number\ of\ infected\ wounds}{Total\ number\ of\ fruits\ per\ treatment} \times 100 \quad \text{(Eq. 1)}$$

Disease severity was assessed as lesion diameter. Lesion diameter (mm) of fruits was measured on 0, 1, 2, 4, 6, and 8 days, and expressed as the average of three readings from each fruit, using Eq. 2:

$$Severity\ index\ (\%) = \frac{Diameter\ of\ infected\ wound}{Total\ number\ of\ fruits\ per\ treatment} \times 100 \quad \text{(Eq. 2)}$$

Physicochemical analyses

Sample preparation

Papaya fruits were first seeded and peeled. Using a juice extractor (Breville Juice Extractor BRE-JE95, Sydney, Australia), the pulp of papaya was puréed and centrifuged (Avanti J-26S Centrifuge, Indianapolis, USA) at 12,000 rpm and 4°C for 10 min. The supernatant was removed into sterile glass vials, and safely stored.

pH

A pH metre (FiveEasy Plus pH meter FP20, Ohio, USA) was used to determine the pH of the centrifuged pulp sample at 25 ± 1°C.

Total soluble solids (TSS)

The TSS was determined using a digital brix refractometer (Milwaukee Instruments, MA871, Swedesboro, U.S.A) at 25 ± 1°C, and reported in °Brix.

Weight loss

At the start of the experiment and at the end of each storage period, three fruits were examined using a digital balance (Ohaus™ Pioneer™ PX 3202/E Precision Balance, Hampton, U.S.A). The results were expressed as the percentage loss of initial weight.

Titratable acidity (TA)

Centrifuged pulp sample (5 mL) was diluted with distilled water to a final volume of 100 mL. Using phenolphthalein as an indicator, the solution was titrated against 0.1 N sodium hydroxide to a pale pink endpoint (colour should last at least 15 s). The volume of sodium hydroxide used to titrate the solution was recorded and converted to grams of citric acid per 100 g pulp (Ducamp-Collin *et al.*, 2008).

Ascorbic acid and total carotenoid contents

The ascorbic acid content of samples was determined using a titration method proposed by Ranganna (1997). Papaya pulp sample (10 g) was powdered under liquid nitrogen, and subsequently added to 100 mL 3% metaphosphoric acid. The extract was filtered through Whatman No. 1 filter paper, and subjected to titration with a standardised solution. The filtrate was titrated until a pink endpoint was reached (colour should last at least 15 s). The results were given in milligrams of ascorbic acid per 100 g pulp.

According to Lee *et al.* (2001), carotenoids were extracted with slight modifications, while total

carotenoid content was established according to Scott (2001). Pulp sample (10 mL) was homogenised (Omni Mixer Homogenizer, CT, USA) for 30 s at speed 4 using 20 mL of extracting solvent. The solvent for extraction contained 10 mL of hexane (Baker Analyzed), 5 mL of acetone (Systerm), and 5 mL of ethanol (Merck). The mixture was centrifuged (Beckman J2-MI Centrifuge, California) for 10 min at 6,500 rpm and 5°C. The top layer of hexane containing carotenoids (yellow) was extracted and adjusted to 10 mL with fresh hexane. Then, absorbance was measured at 450 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank (hexane). The results obtained were expressed as milligrams per 100 mL pulp.

Antioxidant activity

Sample preparation

The antioxidant extraction was conducted following Xu *et al.* (2008) with slight modifications. The pulp sample was ground in liquid nitrogen until powdered, and transferred to 80% methanol in a Falcon tube with equal volumes. The tubes containing the mixture were placed on the rotary shaker (OrbiCult™ Ambient Shaker, Changi, Singapore) at 250 rpm for 30 min at room temperature (25 ± 2°C), and then centrifuged for 15 min using a refrigerated centrifuge (Avanti J-26S Centrifuge, Indianapolis, USA). The supernatant obtained was collected and kept in 2.0 mL Eppendorf tubes at -20°C. The samples were used for subsequent antioxidant analysis.

Total phenolic content (TPC)

The TPC of samples was determined using a modified Folin-Ciocalteu assay (Bae and Suh, 2007). In 1.5 mL microcentrifuge tube, 10 µL of sample extract or gallic acid standard solution was combined with 790 µL of SDW and 50 µL of Folin-Ciocalteu reagent (Sigma-Aldrich). After 1 min, 150 µL of 20% sodium carbonate solution was added, and the solution was mixed by inverting the tubes. The mixture was left at room temperature (25 ± 1°C) for 120 min in the dark. The absorbance was determined at 750 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank (replace juice extract with SDW). A standard curve of gallic acid (y = 0.0059x, R^2 = 0.9967) was then plotted, and the result was represented as mg of gallic acid equivalent (GAE) per /100 mL of sample extract.

Total antioxidant capacity (TAC)

Using the phosphomolybdenum technique, the TAC of pulp sample extracts was determined (Prieto *et al.*, 1999). In 1.5 mL microcentrifuge tube containing 1 mL of reagent solution, 100 µL of sample extract or ascorbic acid standard solution was added. Equal volumes of 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate were then added to make up the reagent solution. The tubes were incubated at 95°C for 90 min in a water bath (Memmert, Germany) in the dark. After cooling to room temperature (25 ± 1°C), absorbance was measured at 695 nm against a prepared blank (UV200-RS Spectrophotometer, MRC, Israel). Sample extract was replaced with SDW for blank. The results were expressed as micrograms ascorbic acid equivalent (AAE) per gram of sample extract, using a standard curve of ascorbic acid (y = 0.0015x, R^2 = 0.9991).

Total flavonoid content (TFC)

The TFC in sample extracts was determined based on the aluminium chloride colorimetric method described by Sakanaka *et al.* (2005). In a test tube, 250 µL of sample extract or catechin standard solution was combined with 1.25 mL of SDW and 75 µL of a 5% sodium nitrite solution. After 5 min of incubation at room temperature (25 ± 1°C), 150 µL of a 10% aluminium chloride solution was added to the mixture. After allowing the mixture to stand for 5 min, 500 µL of 1 N sodium hydroxide was added. The mixture was brought to a volume of 2.5 mL using SDW, and vortexed. Absorbance was determined at 510 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank (juice extract was replaced with SDW). A standard curve of catechin (y = 0.0145x, R^2 = 0.9968) was plotted between from 0 to 100 mg/100 mL. Results were reported as milligrams of catechin equivalent (CE) per 100 mL sample extract.

Primary metabolite analysis

Samples collected at days 0, 4, and 8 were used for the differential primary metabolic profiling analysis. Sample (300 mg) was extracted in 2,700 µL of methanol as described by Roessner-Tunali *et al.* (2003). Next, 300 µL of 0.2 mg/mL ribitol in water was added as a quantification internal standard. Each sample (1 µL) was injected into the gas chromatograph system (Agilent 7200 QTOF-

GC/MS) through a fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm) DB-5 MS stationary phase. The injector temperature was 250°C, with a carrier gas flow rate of 1.0 mL/min. The column temperature was held at 100°C for 1 min; increased to 184°C at a rate of 3°C/min, increased to 190°C at a rate of 0.5°C/min, and increased to 280°C at 15°C/min. The flow rate of the carrier helium (99.999%) gas was 1 mL/min. The following were MS operating parameters: ionisation voltage, 70 eV (electron impact ionisation); ion source temperature.

Statistical analysis

The data collected were analysed statistically using the SPSS 22.0 software (SPSS Inc., IBM). The investigations were conducted in triplicates where three fruits were used for each treatment and control. Results were reported as mean values with standard error (SE) (n = 9). Analysis of variance (One-way ANOVA) was used to detect the significant differences between the mean values of samples using Tukey's honestly significant difference (HSD) test with a significance level of $p < 0.05$.

Results and discussion

Disease incidence and disease severity

HWT had an effect on the disease incidence and disease severity of anthracnose in the papaya fruits. The HWT and storage period had an effect on the incidence of anthracnose in papaya fruits as illustrated in Table 2 ($p < 0.05$).

Table 2. Mean values ± standard deviation of disease incidence, severity, TSS, pH, titratable acidity, and weight loss in control and treated papaya fruits during storage.

Treatment	Incidence of disease (%)	Percent severity index (%)	TSS (°Brix)	pH	Titratable acidity (%)	Weight loss (%)
Day 0						
Control	NA	NA	11.20 ± 0.05^{f}	6.08 ± 0.03^{h}	0.32 ± 0.04^{d}	NA
HWT-CG	NA	NA	10.77 ± 0.08^{bc}	5.82 ± 0.01^{d}	0.28 ± 0.06^{cd}	NA
HWT-DW	NA	NA	10.80 ± 0.06^{b}	5.65 ± 0.01^{g}	0.26 ± 0.04^{d}	NA
Day 1						
Control	NA	NA	11.00 ± 0.58^{ef}	5.52 ± 0.01^{e}	0.21 ± 0.04^{abcd}	0.69 ± 0.27^{a}
HWT-CG	NA	NA	12.13 ± 0.03^{i}	5.23 ± 0.01^{b}	0.17 ± 0.02^{abc}	0.78 ± 0.31^{a}
HWT-DW	NA	NA	13.20 ± 0.06^{k}	5.39 ± 0.02^{cd}	0.17 ± 0.04^{bcd}	0.99 ± 0.02^{a}
Day 2						
Control	33.3 ± 5.36^{a}	NA	11.17 ± 0.03^{ef}	5.49 ± 0.03^{de}	0.19 ± 0.04^{abc}	1.29 ± 0.30^{ab}
HWT-CG	NA	NA	11.50 ± 0.06^{g}	5.52 ± 0.01^{e}	0.13 ± 0.04^{a}	1.30 ± 0.25^{ab}
HWT-DW	NA	NA	11.54 ± 0.06^{g}	5.43 ± 0.01^{i}	0.15 ± 0.04^{abc}	1.71 ± 0.04^{abc}
Day 4						
Control	67.7 ± 3.48^{b}	3.0 ± 5.19^{a}	11.17 ± 0.03^{ef}	5.01 ± 0.05^{a}	0.17 ± 0.04^{abc}	2.42 ± 0.36^{cde}
HWT-CG	33.3 ± 3.67^{a}	NA	10.90 ± 0.06^{cd}	5.88 ± 0.01^{g}	0.19 ± 0.04^{abc}	2.20 ± 0.12^{bcd}
HWT-DW	NA	NA	11.37 ± 0.06^{f}	5.42 ± 0.02^{de}	0.13 ± 0.04^{abc}	3.00 ± 0.07^{de}
Day 6						
Control	67.7 ± 5.48^{b}	7.0 ± 5.19^{b}	10.30 ± 0.06^{a}	5.23 ± 0.01^{b}	0.17 ± 0.02^{abc}	3.88 ± 0.38^{fg}
HWT-CG	67.7 ± 3.67^{b}	4.0 ± 6.15^{a}	12.23 ± 0.08^{i}	5.66 ± 0.02^{f}	0.19 ± 0.04^{abc}	3.29 ± 0.24^{ef}
HWT-DW	33.3 ± 4.63^{a}	2.0 ± 4.08^{a}	12.37 ± 0.06^{j}	5.29 ± 0.02^{bc}	0.15 ± 0.04^{abc}	4.47 ± 0.36^{fg}
Day 8						
Control	100.0 ± 4.48^{c}	22.0 ± 3.19^{c}	11.87 ± 0.03^{h}	5.19 ± 0.01^{b}	0.15 ± 0.02^{bc}	5.80 ± 0.50^{h}
HWT-CG	67.7 ± 3.67^{b}	9.0 ± 6.15^{b}	11.20 ± 0.06^{f}	5.47 ± 0.02^{de}	0.11 ± 0.02^{a}	4.79 ± 0.22^{g}
HWT-DW	33.3 ± 4.63^{a}	7.0 ± 4.08^{b}	10.23 ± 0.06^{a}	5.02 ± 0.05^{a}	0.10 ± 0.01^{a}	6.49 ± 0.50^{h}

HWT: hot water treatment; HWT-CG: hot water treated fruits inoculated with *C. gloeosporioides*; and HWT-DW: hot water treated fruits inoculated with distilled water.

Anthracnose incidence was indicated by the presence of symptoms such as tiny, sunken, and water-soaked lesions (Sivakumar *et al.*, 2002a). On days 0 and 1, all the fruits were free of symptoms; however, on day 2, control fruits began to show symptoms. On day 4, almost half of the control fruits showed symptoms, while HWT-CG showed symptoms.

Meanwhile, the HWT-DW remained symptom-free. On day 8, The disease incidence of the control and treated papaya fruits differed significantly. All the control fruits showed symptoms of the disease, while only 50% of HWT-CG samples showed symptoms. The HWT-DW showed significantly less disease incidence, with only a third of the papaya fruits showing disease symptoms. According to Ayón-Reyna *et al.* (2017b), HWT fruits had a significantly lower disease incidence than control fruits. The severity of anthracnose on the papaya fruits was dependant on the time of storage and HWT ($p < 0.05$). Table 2 shows the disease severity as evaluated by measuring the lesion diameter (Sivakumar *et al.*, 2002b). The disease severity of HWT fruits was significantly reduced as compared to control fruits. On the final day of storage, the control fruits presented a significantly higher disease severity of 22 mm than HWT-CG (9 mm) and HWT-DW (7 mm). It was reported that the severity of anthracnose was significantly lowered in HWT papaya fruits as compared to control fruits (Vilaplana *et al.*, 2020).

According to Li *et al.* (2013b), the mechanism of action of hot water treatment against anthracnose minimises the carrier rate of *C. gloeosporioides* in fruit peel, which significantly reduced anthracnose incidence of stem-end rot, thus successfully delaying fruit softening but moderately promoting the rate of fruit colouring. HWT decreased anthracnose index and maturity of fruit to some degree, and prompted changes in wax arrangements on the treated fruit surface, thus causing the wax to melt. The melted wax created by HWT is supposed to have completely or partially closed gaps in the epidermis, which could prevent the pathogen from invading physically, hence limiting the risk of disease. Both of these affect the pathogens directly, and the host indirectly (Strano *et al.*, 2014). Based on some findings, non-lethal heat treatment stresses fruit somewhat, thus eliciting a temporary pause in normal metabolism that resolves when the fruit is brought back to a non-stressful temperature (Fallik, 2004). The effect is a postponement of ripening and softening of the fruit that has been treated with hot water (Liu *et al.*, 2020).

Physicochemical analyses

Sweet taste is a critical parameter of fruit quality. Sucrose, glucose, and fructose concentrations are utilised as a ripening index to determine the stage of ripeness and papaya quality. TSS is an indicator of sweetness routinely used for evaluating the quality of fruits. Values for TSS (10.2 to 13.2 °Brix) of samples were in the range of desirable qualities for Eksotika II papaya (Azhar *et al.*, 2020). Based on Table 2, the highest reading was observed on day 1 for HWT-DW, which was 13.2 °Brix, and the lowest on day 8 for HWT-DW, which was 10.2 °Brix. The results obtained were in tandem with Arina *et al.* (2009) who reported that the TSS of HWT and untreated papayas did not significantly change throughout the ripening period. Li *et al.* (2013a) also concluded that heat treatment did not affect the TSS contents of 'Red Fuji' apples.

The pH of both HWT samples increased after the second day of storage, and remained higher than the control until day 6, as shown in Table 2. On the final day of storage, HWT-DW pH decreased slightly below that of the control samples. The value of the pH did not significantly change between the control and hot water treated samples. These findings were in accordance with Arina *et al.* (2009) and Vilaplana *et al.* (2020) who reported pH values between 5.4 - 5.7 for both treated and untreated fruits.

During the postharvest period, climacteric fresh fruits lose weight primarily due to transpiration and respiration (Kader and Ben-Yehoshua, 2000). The quality of papaya fruits could be determined by measuring the weight loss (Table 2). No significant weight loss was measured among the HWT samples as compared to control samples. Similar trends were observed in hot water treated papaya (*Carica papaya* cv. 'Maradol') and strawberry (*Fragaria* × *ananasa* Duch), where the treated fruits did not show significant weight loss differences ($p > 0.05$) in comparison to the control fruits throughout the storage period (Chávez-Sánchez *et al.*, 2013; Contigiani *et al.*, 2020).

The TA values (Table 2) of all samples decreased gradually throughout storage. The TA of both hot water treated samples remained significantly lower than the control samples on the last day of

storage, with values of 0.107% for HWT-CG and 0.102% for HWT-DW, as compared to 0.149% of control. Rabiei *et al*. (2011) reported a significantly lower TA in heat-treated than unheated 'Red Delicious' apples, consistent with the results obtained. Similarly, Shao *et al*. (2012) showed that the TA content of 'Gala' apple fruit reduced during storage. The first decline in TA following HWT was comparable to the previously observed drop in TA following short-term temperature fluctuations (0, 5, 10, 15, or 20°C) (Proulx *et al*., 2005). This could speed up the ripening metabolic processes that deplete organic acids (Rashid *et al.,* 2015). Based on all the physicochemical parameters examined, there was no significant change in fruit quality between control and treated fruits, except for TA. This further supported the view that HWT would not impair the important qualities of the fruits.

Ascorbic acid and total carotenoid contents

Ascorbic acid has strong antioxidant properties, and the colour of the fruits is determined by the number of carotenoids present (da Silva Junior *et al.,* 2018). The ascorbic acid content decreased from day 0 to 1; however, it increased gradually throughout the storage period (Table 3). The ascorbic acid content of treated samples remained significantly higher than control samples on day 8. In similar findings, *Penicillium expansum*, *Botrytis cinerea*, or *Colletotrichum gloeosporioides* infections were reported in apples; the fruits with the lowest decay region contained the highest ascorbic acid (Sharma *et al*., 2013). By inactivating enzymes such as ascorbate oxidase, heat treatment stabilised the ascorbic acid (Leong and Oey, 2014).

Table 3. Mean values ± standard deviation of the effect of hot water treatment (HWT) on ascorbic acid content and total carotenoid content.

Treatment	Ascorbic acid content (mg/100 mL)	Total carotenoid content (mg/100 mL)
Day 0		
Control	42.24 ± 3.50^{c}	0.90 ± 0.15^{ef}
HWT-CG	52.92 ± 4.12^{cd}	0.80 ± 0.15^{def}
HWT-DW	50.43 ± 3.90^{b}	0.85 ± 0.15^{ef}
Day 1		
Control	40.65 ± 3.53^{b}	0.78 ± 1.49^{def}
HWT-CG	60.87 ± 1.33^{e}	0.72 ± 0.15^{cde}
HWT-DW	52.92 ± 4.12^{d}	0.69 ± 0.15^{cde}
Day 2		
Control	40.65 ± 3.46^{b}	0.65 ± 2.46^{cde}
HWT-CG	62.54 ± 4.21^{e}	0.68 ± 0.15^{cde}
HWT-DW	51.56 ± 3.46^{bc}	0.64 ± 0.15^{cde}
Day 4		
Control	42.24 ± 3.79^{c}	0.60 ± 0.15^{cd}
HWT-CG	64.28 ± 4.00^{d}	0.58 ± 0.15^{bc}
HWT-DW	51.79 ± 3.99^{bc}	0.57 ± 0.15^{bc}
Day 6		
Control	43.83 ± 1.33^{d}	0.54 ± 0.23^{bc}
HWT-CG	62.47 ± 2.67^{bcd}	0.55 ± 0.15^{bc}
HWT-DW	52.15 ± 3.53^{bc}	0.53 ± 0.15^{bc}
Day 8		
Control	42.92 ± 2.31^{cd}	0.48 ± 0.15^{a}
HWT-CG	66.21 ± 1.33^{ef}	0.45 ± 0.15^{a}
HWT-DW	55.09 ± 3.53^{e}	0.47 ± 0.30^{a}

HWT: hot water treatment; HWT-CG: hot water treated fruits inoculated with *C. gloeosporioides*); and HWT-DW: hot water treated fruits inoculated with distilled water.

The total carotenoid content showed a decreasing trend throughout the storage period (Table 3). The highest recorded value was on Day 0 (0.9 µg/100 mL) from the control sample, and the lowest was recorded on Day 8 (0.45 µg/100 mL) from the HWT-CG samples. According to Supapvanich and Promyou (2017), both hot water dip and the control samples did not enhance the carotenoid contents of 'Holland' papaya fruit, and it remained constant throughout storage. Furthermore, the decrease in total carotenoid content could be due to the oxidative reactions during storage (Charan *et al.*, 2017).

Antioxidant activity (total phenolic content, total antioxidant capacity, and total flavonoid content)

The antioxidant activity of papaya fruits was measured by Folin-Ciocalteu, phosphomolybdenum, and aluminium chloride colorimetric assays. The TPC (Table 4) showed a gradual increase until day 4, after which it decreased significantly on day 6, and increased slightly on day 8. The highest value was recorded by HWT-DW on day 4 with a value of 50.77 mg GAE/100 mL, while the lowest (16.94 mg GAE/100 ml) was on day 1 for HWC-CG. The rapid oxidation of phenolics could be attributable to the faster water loss and higher enzyme activity in the pulp kept at room temperature (Jiang and Fu, 1999). Consequently, in the presence of molecular oxygen, polyphenol oxidase (PPO) could interact with phenolics and catalyse their oxidation to quinones (Huang *et al.*, 2005). Consequently, as storage time increases, the overall polyphenol content decreases.

Table 4. Mean values ± standard deviation of effect of hot water treatment (HWT) on total phenolic content, total antioxidant capacity, and total flavonoid content.

Treatment	Total phenolic content (mg GAE/100 mL)	Total antioxidant capacity (µg AAE/mL)	Total flavonoid content (mg CE/ 100 mL)
Day 0			
Control	26.31 ± 0.51^{bc}	263.52 ± 12.46^{cd}	0.62 ± 0.05^{ab}
HWT-CG	24.05 ± 0.24^{b}	247.59 ± 4.44^{b}	0.79 ± 0.04^{c}
HWT-DW	29.64 ± 0.45^{bc}	269.25 ± 2.36^{cd}	0.74 ± 0.05^{ab}
Day 1			
Control	24.29 ± 0.87^{b}	255.28 ± 13.11^{c}	0.59 ± 0.02^{ab}
HWT-CG	16.96 ± 0.31^{a}	242.80 ± 3.45^{b}	0.72 ± 0.09^{ab}
HWT-DW	38.63 ± 0.94^{d}	230.23 ± 4.02^{b}	0.66 ± 0.04^{b}
Day 2			
Control	25.12 ± 0.57^{b}	239.26 ± 4.06^{b}	0.69 ± 0.10^{c}
HWT-CG	34.64 ± 1.29^{c}	236.48 ± 7.72^{b}	0.76 ± 0.10^{b}
HWT-DW	29.05 ± 0.26^{bc}	239.44 ± 7.50^{bc}	0.75 ± 0.09^{b}
Day 4			
Control	40.18 ± 0.98^{d}	253.52 ± 6.30^{c}	0.62 ± 0.07^{a}
HWT-CG	29.46 ± 0.37^{bc}	290.00 ± 19.32^{d}	0.69 ± 0.08^{a}
HWT-DW	50.77 ± 0.36^{e}	282.96 ± 2.91^{d}	0.62 ± 0.02^{a}
Day 6			
Control	27.62 ± 0.30^{bc}	270.19 ± 13.69^{d}	0.85 ± 0.08^{d}
HWT-CG	27.44 ± 0.46^{bc}	298.89 ± 7.47^{d}	0.88 ± 0.08^{cd}
HWT-DW	30.42 ± 0.98^{c}	275.93 ± 6.05^{d}	0.85 ± 0.12^{cd}
Day 8			
Control	26.19 ± 0.16^{bc}	239.44 ± 12.06^{b}	0.90 ± 0.08^{c}
HWT-CG	32.14 ± 0.45^{c}	244.07 ± 7.96^{b}	0.98 ± 0.02^{d}
HWT-DW	36.73 ± 0.68^{cd}	192.96 ± 5.38^{a}	0.97 ± 0.03^{e}

HWT: hot water treatment; HWT-CG: hot water treated fruits inoculated with *C. gloeosporioides*; and HWT-DW: hot water treated fruits inoculated with distilled water.

The TAC showed no significant difference for day 0 to 2 (Table 4). However, the TAC of treated samples increased from day 4 to the final day of storage. HWC-CG recorded the highest value (298.89 µg AAE/mL) on day 6, while the lowest value (192.96 µg AAE/mL) was recorded by HWT-DW on day 8. However, antioxidant activity varied with the ripening stage as shown in Table 4; the TFC showed no significant increase in the first four days of storage. However, the TPC began to increase on day 6. The TFC peaked on day 8 with 1.423 and 1.926 mg CE/100 mL for HWT-CG and HWT-DW, respectively.

Analyses of physicochemical parameters (pH, TSS, TA, and weight loss) of papaya samples after treatment were conducted to guarantee that the HWT did not affect the eating quality of the papaya. Moreover, these characteristics contribute to the flavour and texture of the fruit as a whole. TSS assesses the sweetness, pH and TA measure the overall acidity or sourness of the fruit, and weight loss relates to water loss, which is an important issue that must be accounted for during long-distance storage and shipping.

Once the fruit has been harvested, its stored water and nutrients such as sugars and organic acids continuously deplete by transpiration and respiration to provide energy and other metabolic intermediates for maintaining cellular processes and biological activities (Ma *et al*., 2021). Furthermore, if by-products of reactive oxygen species (ROS) created in pathways of mitochondrial respiration reactions are not efficiently scavenged, lipid peroxidation, membrane disintegration, protein and nucleic acid damage, and fruit senescence would occur (Tian *et al*., 2013). This induces the synthesis of phenolics and flavonoids by activating the fruit's defence mechanism against ROS. The high TPC on day 4 in the HWT fruits could be a result from the HWT which released bound phenolics and inactivated polyphenol oxidase (Musilova *et al.*, 2020). On day 8, the slight increase in the TPC was correlated with the increase in fungal activity which triggered a defence mechanism (Fuerst *et al.*, 2014). As a defence mechanism, plant tissues generate phenolics (Laura *et al.*, 2019), thus raising their levels in response to stress such as fungal infection damage or unfavourable conditions of environment (*e.g*., hot climate) (Mikulic-Petkovsek *et al.*, 2014). Rienth *et al.* (2014) reported that stress caused by fruit heating and phenolic synthesis was triggered. Additionally, several investigations have demonstrated that HWT boosted the expression of enzymes involved in the manufacture of phenolic compounds (Trinh *et al.*, 2018). Phenolic compounds are substrates of fungitoxic quinones causing oxidative reactions, and phenolic-accumulating plant cells have become an unfavourable environment for the growth of pathogens (Avasthi *et al.*, 2018). This rise could be a result of the stress caused by heat treatment, as this state is critical for the activation and accumulation of phenolics (Jacobo-Velázquez *et al.*, 2011). Plants, on the whole, respond quickly to increased ROS (reactive oxygen species) produced by abiotic stresses like changes in storage temperature (Vickers *et al.*, 2009). These modifications result in a rise in the activity of cellular antioxidants, which in turn increases the activity of natural antioxidants (Mittler, 2002). By treating fruit with hot water and delaying ripening, we can increase antioxidant molecules including AA (ascorbic acid), GSH (glutathione), and free phenolics and flavonoids (Sharma *et al.*, 2015).

A close relationship between TPC and antioxidant activity in selected fruits, vegetables, and grain products has also been documented by several authors (Ramkissoon *et al.*, 2012). Phenolic compounds have the propensity to donate hydrogen or electrons, and form stable radical intermediates, which makes them excellent antioxidants (Vuolo *et al.*, 2019).

Primary metabolite analysis

Different accumulated metabolites were studied to investigate the changes in metabolic composition between HWT and control fruits. The biochemical changes were determined by GC-MS. A total of 52 identified metabolites were monitored in the same sample sets using GC-MS. After conducting an analysis of significance with ANOVA ($p < 0.05$), 17 detected metabolites, mostly fatty acids, were found to be significantly up- or downregulated in HWT fruits as compared to control fruits with the same storage period (Table 5).

Primary metabolites are directly involved in normal growth, development, and reproduction. In the present work, the content of ornithine, oleic acid, and tetradecanoic acid increased in heat treated pericarp as compared to the control during storage, and levels of seven types of sugars increased in heat treated pericarp as compared to the control at 2 h after

Table 5. Differential accumulated primary metabolites in hot water treated fruits as compared to control fruits.

Compound	Day 0	Day 4	Day 8
Alcohol			
Glycerine	1.04*	1.01*	1.15*
Fatty acid			
Pentadecanoic acid	0.26	0.328	
n-Hexadecanoic acid	1.25*	UP	0.92
Octadecanoic acid	1.61*	DOWN	0.66
11,14,17-Eicosatrienoic acid		DOWN	
Eicosanoic acid		DOWN	
9,12,15-Octadecatrienoic acid	1.32*	UP	0.62
Other			
Hydrazine, 1,2-dimethyl-	1.12*	0.98	0.98
O-Butylisourea	DOWN	UP	
Isopropyl myristate (ester)	1.11*	1.43*	0.89
9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	1.74*		0.71
1,2-Cyclopentanedione		DOWN	DOWN
2-Butanone, 4-hydroxy-3-methyl-		UP	2.48*
1,2-Cyclopentanedione			DOWN
1H-Pyrazole, 1,5-dimethyl-	UP		
2(3H)-Furanone, 5-methyl-			UP
Benzophenone			UP

The table shows the ratio (HWT/control during the same period of storage). *Significant difference ($p < 0.05$). UP: metabolite was detected in the HWT pulp but not in the control pulp.

HWT (Table 5). In plants, ornithine is required for the synthesis of polyamines and alkaloids, which contribute to oxidative stress tolerance in plants subjected to severe water stress (Roessner-Tunali *et al.*, 2003).

Conclusion

Anthracnose disease caused by *Colletotrichum gloeosporioides* affected the quality and storability of papaya fruits. Fungi infecting papaya fruits developed symptoms of anthracnose disease which led to fruit decay and loss of fruit quality. All hot water treated papaya fruits (HWT-CG and HWT-DW) exhibited lower disease incidence and severity throughout postharvest storage as compared to control papaya fruits. In comparison to papaya fruits that were not treated with hot water (control), papaya fruits that were treated with hot water preserved their physicochemical features, and had a higher total flavonoid and phenolic contents, as well as an increase in stress-related metabolites.

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References

Arina, M. A., Boyce, A. N. and Chandran, S. 2009. Effects of postharvest hot water treatment on physiological and biochemical properties of 'Eksotika' papaya during ripening. In Southeast Asia Symposium on Quality and Safety of Fresh and Fresh-Cut Produce. Bangkok, Thailand.

Avasthi, S., Gautam, A. K. and Bhaduaria, R. 2018. Changes in biochemical components in *Aloe*

vera (L.) Burm. f. leaves infected with *Fusarium proliferatum* (Matsushima) Nirenberg. Journal of Applied Research on Medicinal and Aromatic Plants 11: 61-66.

Ayón-Reyna, L. E., González-Robles, A., Rendón-Maldonado, J. G., Báez-Flores, M. E., López-López, M. E. and Vega-García, M. O. 2017a. Application of a hydrothermal-calcium chloride treatment to inhibit postharvest anthracnose development in papaya. Postharvest Biology and Technology 124: 85-90.

Ayón-Reyna, L. E., López-Valenzuela, J. Á., Delgado-Vargas, F., López-López, M. E., Molina-Corral, F. J., Carrillo-López, A. and Vega-García, M. O. 2017b. Effect of the combination hot water-calcium chloride on the *in vitro* growth of *Colletotrichum gloeosporioides* and the postharvest quality of infected papaya. The Plant Pathology Journal 33(6): 572.

Azhar, H. M., Johari, S., Sulastri, J. N., Razali, M., Zulfa, M. M., Faimah, G. N. and Mariatulqabtiah, A. R. 2020. Field performance of selected papaya hybrids for tolerance to dieback disease. Journal of Tropical Agriculture and Food Science 48(1): 25-33.

Bae, S.-H. and Suh, H.-J. 2007. Antioxidant activities of five different mulberry cultivars in Korea. LWT – Food Science and Technology 40(6): 955-962.

Batista, D. P., Souza, R. D. S., Santos-Magalhães, N. S., de Sena-Filho, J. G., Teodoro, A. V., Grillo, L. A. and Dornelas, C. B. 2014. Polymer/layered silicate nanocomposite as matrix for bioinsecticide formulation. In Macromolecular Symposia 344(1): 14-21.

Bravo, L. 1998. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutrition Reviews 56(11): 317-333.

Charan, S. M., Gomez, S., Sheela, K. B., Pushpalatha, P. B. and Suman, K. T. 2017. Effect of storage conditions and duration on quality of passion fruit (*Passiflora edulis* Sims.) nectar. Asian Journal of Dairy and Food Research 36(2): 161-165.

Chávez-Sánchez, I., Carrillo-López, A., Vega-García, M. and Yahia, E. M. 2013. The effect of antifungal hot-water treatments on papaya postharvest quality and activity of pectinmethylesterase and polygalacturonase. Journal of Food Science and Technology 50(1): 101-107.

Contigiani, E. V., Jaramillo Sánchez, G. M., Castro, M. A., Gómez, P. L. and Alzamora, S. M. 2020. Efficacy of mild thermal and pulsed light treatments, individually applied or in combination, for maintaining postharvest quality of strawberry cv. Albion. Journal of Food Processing and Preservation 2020: e15095.

da Silva Junior, E. V., de Melo, L. L., de Medeiros, R. A. B., Barros, Z. M. P. and Azoubel, P. M. 2018. Influence of ultrasound and vacuum assisted drying on papaya quality parameters. LWT – Food Science and Technology 97: 317-322.

Damm, U., Cannon, P. F., Woudenberg, J. H. C., Johnston, P. R., Weir, B. S., Tan, Y. P., ... and Crous, P. W. 2012. The *Colletotrichum boninense* species complex. Studies in Mycology 73: 1-36.

Di Francesco, A., Mari, M. and Roberti, R. 2018. Defense response against postharvest pathogens in hot water treated apples. Scientia Horticulturae 227: 181-186.

Ducamp-Collin, M. N., Ramarson, H., Lebrun, M., Self, G. and Reynes, M. 2008. Effect of citric acid and chitosan on maintaining red colouration of litchi fruit pericarp. Postharvest Biology and Technology 49(2): 241-246.

Fallik, E. 2004. Prestorage hot water treatments (immersion, rinsing and brushing). Postharvest Biology and Technology 32(2): 125-134.

Food and Agriculture Organisation (FAO). 2019. FAOSTAT. Retrieved from FAO website: http://faostat.fao.org

Fuerst, E. P., Okubara, P. A., Anderson, J. V. and Morris, C. F. 2014. Polyphenol oxidase as a biochemical seed defense mechanism. Frontiers in Plant Science 5: 689.

Gao, Q., Tan, Q., Song, Z., Chen, W., Li, X. and Zhu, X. 2020. Calcium chloride postharvest treatment delays the ripening and softening of papaya fruit. Journal of Food Processing and Preservation 44(8): e14604.

Huang, X. M., Wang, H. C., Yuan, W. Q., Lu, J. M., Yin, J. H., Luo, S. and Huang, H. B. 2005. A

study of rapid senescence of detached litchi: Roles of water loss and calcium. Postharvest Biology and Technology 36(2): 177-189.

Jacobo-Velázquez, D. A., Martínez-Hernández, G. B., del C. Rodríguez, S., Cao, C. M. and Cisneros-Zevallos, L. 2011. Plants as biofactories: Physiological role of reactive oxygen species on the accumulation of phenolic antioxidants in carrot tissue under wounding and hyperoxia stress. Journal of Agricultural and Food Chemistry 59(12): 6583-6593.

Jiang, Y. M. and Fu, J. R. 1999. Postharvest browning of litchi fruit by water loss and its prevention by controlled atmosphere storage at high relative humidity. LWT – Food Science and Technology 32(5): 278-283.

Kader, A. A. and Ben-Yehoshua, S. 2000. Effects of superatmospheric oxygen levels on postharvest physiology and quality of fresh fruits and vegetables. Postharvest Biology and Technology 20(1): 1-13.

Lam, P. F. and Zaipun, M. Z. 1987. Changes associated with different stages of harvesting and ripening of Eksotika papaya at ambient temperatures. MARDI Research Bulletin 15(1): 21-26.

Laura, A., Moreno-Escamilla, J. O., Rodrigo-García, J. and Alvarez-Parrilla, E. 2019. Phenolic compounds. In Yahia, E. and Carrillo-Lopez, A. (eds). Postharvest Physiology and Biochemistry of Fruits and Vegetables, p. 253-271. United States: Woodhead Publishing.

Lee, H., Castle, W. S. and Coates, G. A. 2001. High-performance liquid chromatography for the characterisation of carotenoids in the new sweet orange (Earlygold) grown in Florida, USA. Journal of Chromatography A 913(1-2): 371-377.

Leong, S. Y. and Oey, I. 2014. Effect of pulsed electric field treatment on enzyme kinetics and thermostability of endogenous ascorbic acid oxidase in carrots (*Daucus carota* cv. Nantes). Food Chemistry 146: 538-547.

Li, L., Li, X., Wang, A., Jiang, Y. and Ban, Z. 2013a. Effect of heat treatment on physiochemical, colour, antioxidant and microstructural characteristics of apples during storage. International Journal of Food Science and Technology 48(4): 727-734.

Li, X., Zhu, X., Zhao, N., Fu, D., Li, J., Chen, W. and Chen, W. 2013b. Effects of hot water treatment on anthracnose disease in papaya fruit and its possible mechanism. Postharvest Biology and Technology 86: 437-446.

Liu, S., Huang, H., Huber, D. J., Pan, Y., Shi, X. and Zhang, Z. 2020. Delay of ripening and softening in 'Guifei' mango fruit by postharvest application of melatonin. Postharvest Biology and Technology 163: 111136.

Ma, Q., Lin, X., Wei, Q., Yang, X., Zhang, Y. N. and Chen, J. 2021. Melatonin treatment delays postharvest senescence and maintains the organoleptic quality of 'Newhall' navel orange (*Citrus sinensis* (L.) Osbeck) by inhibiting respiration and enhancing antioxidant capacity. Scientia Horticulturae 286: 110236.

Mikulic-Petkovsek, M., Schmitzer, V., Jakopic, J., Cunja, V., Veberic, R., Munda, A. and Stampar, F. 2014. *Colletotrichum lindemuthianum* infection causes changes in phenolic content of French green bean pods. Scientia Horticulturae 170: 211-218.

Mishra, B. B., Gautam, S., Chander, R. and Sharma, A. 2015. Characterisation of nutritional, organoleptic and functional properties of intermediate moisture shelf stable ready-to-eat *Carica papaya* cubes. Food Bioscience 10: 69-79.

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science 7(9): 405-410.

Musilova, J., Lidikova, J., Vollmannova, A., Frankova, H., Urminska, D., Bojnanska, T. and Toth, T. 2020. Influence of heat treatments on the content of bioactive substances and antioxidant properties of sweet potato (*Ipomoea batatas* L.) tubers. Journal of Food Quality 2020: 8856260.

Prieto, P., Pineta, M. and Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Analytical Biochemistry 269(2): 337-341.

Proulx, E., Cecilia, M., Nunes, N., Emond, J. P. and Brecht, J. K. 2005. Quality attributes limiting papaya postharvest life at chilling and non-chilling temperatures. Proceedings of the

Florida State Horticultural Society 118: 389-395.

Prunier, C., Klein, N. and Neuwald, D. A. 2018. Hot water treatment-using the apples own defence potential. In 18th International Conference on Organic Fruit-Growing: Proceedings of the Conference. Hohenheim, Germany.

Rabiei, V., Es-haghi, S., Aazami, M. A. and Sharafi, Y. 2011. Combined effects of hot air and calcium chloride on quality and antioxidant enzymes activity in 'red delicious' apple fruits. Journal of Medicinal Plants Research 5(19): 4954-4961.

Ramkissoon, J. S., Mahomoodally, M. F., Ahmed, N. and Subratty, A. H. 2012. Relationship between total phenolic content, antioxidant potential, and antiglycation abilities of common culinary herbs and spices. Journal of Medicinal Food 15(12): 1116-1123.

Ranganna, S. 1977. Manual of analysis of fruit and vegetable products (volume 197). India: Tata McGraw-Hill.

Rashid, M. H. A., Grout, B. W. W., Continella, A. and Mahmud, T. M. M. 2015. Low-dose gamma irradiation following hot water immersion of papaya (*Carica papaya* linn.) fruits provides additional control of postharvest fungal infection to extend shelf life. Radiation Physics and Chemistry 110: 77-81.

Rienth, M., Torregrosa, L., Luchaire, N., Chatbanyong, R., Lecourieux, D., Kelly, M. T. and Romieu, C. 2014. Day and night heat stress trigger different transcriptomic responses in green and ripening grapevine (*Vitis vinifera*) fruit. BMC Plant Biology 14(1): 1-18.

Roessner-Tunali, U., Hegemann, B., Lytovchenko, A., Carrari, F., Bruedigam, C., Granot, D. and Fernie, A. R. 2003. Metabolic profiling of transgenic tomato plants overexpressing hexokinase reveals that the influence of hexose phosphorylation diminishes during fruit development. Plant Physiology 133(1): 84-99.

Sakanaka, S., Tachibana, Y. and Okada, Y. 2005. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). Food Chemistry 89(4): 569-575.

Sarkar, A. K. 2016. Anthracnose diseases of some common medicinally important fruit plants. Journal of Medicinal Plants Studies 4(3): 233-236.

Scott, K. J. 2001. Detection and measurement of carotenoids by UV/VIS spectrophotometry. Current Protocols in Food Analytical Chemistry 2001: F2-2.1-F2.2.10.

Shao, X. F., Tu, K., Tu, S. and Tu, J. 2012. A combination of heat treatment and chitosan coating delays ripening and reduces decay in "Gala" apple fruit. Journal of Food Quality 35(2): 83-92.

Sharma, R. R., Singh, D. and Pal, R. K. 2013. Synergistic influence of pre-harvest calcium sprays and postharvest hot water treatment on fruit firmness, decay, bitter pit incidence and postharvest quality of Royal Delicious apples (*Malus x domestica* Borkh). American Journal of Plant Sciences 4(1): 153-159.

Sharma, S., Saxena, D. C. and Riar, C. S. 2015. Antioxidant activity, total phenolics, flavonoids and antinutritional characteristics of germinated foxtail millet (*Setaria italica*). Cogent Food and Agriculture 1(1): 1081728.

Sivakumar, D., Hewarathgamagae, N. K., Wijeratnam, R. W. and Wijesundera, R. L. C. 2002a. Effect of ammonium carbonate and sodium bicarbonate on anthracnose of papaya. Phytoparasitica 30(5): 486-492.

Sivakumar, D., Wijeratnam, R. W., Abeyesekere, M. and Wijesundera, R. L. C. 2002b. Combined effect of Generally Regarded As Safe (GRAS) compounds and *Trichoderma harzianum* on the control of postharvest diseases of rambutan. Phytoparasitica 30(1): 43-51.

Strano, M. C., Calandra, M., Aloisi, V., Rapisarda, P., Strano, T. and Ruberto, G. 2014. Hot water dipping treatments on Tarocco orange fruit and their effects on peel essential oil. Postharvest Biology and Technology 94: 26-34.

Supapvanich, S. and Promyou, S. 2017. Hot water incorporated with salicylic acid dips maintaining physicochemical quality of 'Holland' papaya fruit stored at room temperature. Emirates Journal of Food and Agriculture 29: 18-24.

Talcott, S. T., Moore, J. P., Lounds-Singleton, A. J. and Percival, S. S. 2005. Ripening associated phytochemical changes in mangos (*Mangifera indica*) following thermal quarantine and low-temperature storage. Journal of Food Science 70(5): C337-C341.

Terao, D., de Lima Nechet, K., Frighetto, R. T. S. and Sasaki, F. F. C. 2019. Ozonated water

combined with heat treatment to control the stem-end rot of papaya. Scientia Horticulturae 257: 108722.

Tian, S., Qin, G. and Li, B. 2013. Reactive oxygen species involved in regulating fruit senescence and fungal pathogenicity. Plant Molecular Biology 82(6): 593-602.

Trinh, L. T. P., Choi, Y. S. and Bae, H. J. 2018. Production of phenolic compounds and biosugars from flower resources *via* several extraction processes. Industrial Crops and Products 125: 261-268.

Ummarat, N., Matsumoto, T. K., Wall, M. M. and Seraypheap, K. 2011. Changes in antioxidants and fruit quality in hot water-treated 'Hom Thong' banana fruit during storage. Scientia Horticulturae 130(4): 801-807.

Vickers, C. E., Gershenzon, J., Lerdau, M. T. and Loreto, F. 2009. A unified mechanism of action for volatile isoprenoids in plant abiotic stress. Nature Chemical Biology 5(5): 283-291.

Vilaplana, R., Chicaiza, G., Vaca, C. and Valencia-Chamorro, S. 2020. Combination of hot water treatment and chitosan coating to control anthracnose in papaya (*Carica papaya* L.) during the postharvest period. Crop Protection 128: 105007.

Vilaplana, R., Hurtado, G. and Valencia-Chamorro, S. 2018. Hot water dips elicit disease resistance against anthracnose caused by *Colletotrichum musae* in organic bananas (*Musa acuminata*). LWT – Food Science and Technology 95: 247-254.

Vuolo, M. M., Lima, V. S. and Junior, M. R. M. 2019. Phenolic compounds: Structure, classification, and antioxidant power. In Campos, M. R. S. (ed). Bioactive Compounds - Health Benefits and Potential Applications, p. 33-50. United States: Woodhead Publishing.

Xu, G., Liu, D., Chen, J., Ye, X., Ma, Y. and Shi, J. 2008. Juice components and antioxidant capacity of citrus varieties cultivated in China. Food Chemistry 106(2): 545-551.