# Treatment of Dragonfruit (*Hylocereus polyrhizus*) with Salicylic Acid and Methyl Jasmonate improves Postharvest Physico-chemical Properties and Antioxidant Activity during Cold Storage

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As the market for tropical fruit constantly expands, cold storage is increasingly used for transporting fruits over long distances. This is an economic postharvest tool, yet challenges tropical fruits by exposure to chilling injury. An assessment of the effect of abiotic stresses, induced by cold storage, on dragonfruit was conducted. Dragonfruit was treated with salicylic acid (SA) and methyl jasmonate (MJ) and subjected to cold storage for three weeks. Fruits were treated with either SA or MJ, administered at four different concentrations, along with an untreated control, and stored at 6 °C. Changes in biochemical quality parameters, along with bioactive content and antioxidant activity were assessed during storage. Application of SA was found to reduce the metabolic activity of the fruit, as determined by soluble solids content and titratable acidity. Meanwhile, MJ significantly enhanced the betacyanin content and antioxidant activity. We demonstrate that cold storage can be applied for dragonfruit, by combining the treatment with the application of hormones, especially MJ which can enhance the antioxidant activity of dragonfruit under cold storage.

***Keywords:*** abiotic stress, metabolic activity, ripening, nutraceutical value, stress hormones

***Running title:*** Dragonfruit responses to stress hormones and chilling

## 1. Introduction

Cold storage is an economic postharvest tool for extending shelf life of perishable plant products. However, it is a source of postharvest stress on tropical fruits, which may result in physiological changes in the plant organ (Wang and Frei, 2011). Chilling injury of fruits is closely associated with degradation of the membrane integrity, resulting in oxidation of phenolic compounds. This negatively affects the physical and biochemical qualities of fruits, manifesting through browning and shrivelling of fruit surface, as well as depletion of phenolic compounds.

Phenolic compounds and antioxidants are of particular importance, as they play a role in acclimation to cold stress (Gonzalez-Aguilar et al., 2004). An increase in accumulation of these compounds during chilling tolerance has been reported, which serves to regulate the oxidative stress on the biological system (Chen et al., 2006). This demonstrates an interesting phenomenon where abiotic stresses, such as chilling, can trigger intrinsic defence mechanisms of the plant and ultimately enhance the bioactive content of the fruit. Exploiting these natural resistance mechanisms for extending the shelf life of products is increasingly being explored as an alternative to traditional postharvest tools (Ashgari and Hassanlooe, 2015). The authors have reported this on other tropical fruits such as mangosteen and carambola (Mustafa et al., 2016), and this research will explore this approach on the tropical fruit, dragonfruit.

Dragonfruit (*Hylocerus* sp.) is an exotic fruit of the family Cactaceae commonly found in the tropical regions of South America and Southeast Asia. It has been increasing in popularity due to its attractive purple coloration and distinct nutraceutical health properties and anti-inflammatory characteristics (Macias-Ceja et al., 2016; Dembitsky et al., 2011). The characteristic skin colour is attributed to the presence of betacyanins and the flesh is also rich in betacyanins and phenolic compounds, and is characterized by potent antioxidant activity

(Wu et al., 2006). As a tropical fruit, dragonfruit is best stored at 14 - 20 °C, and is susceptible to chilling injury at 6 °C (Hoa et al., 2006; Nerd et al., 1999). Moreover, the fruit is preferably harvested at maturity, which minimises the incidence of chilling injury during cold storage, allowing the fruit to maintain desirable quality during marketing (Wanitchang et al., 2010; Nerd et al. 1999). Nonetheless, relatively scarce information is available on the changes in the physico-chemical properties and antioxidant activity of the fruit during cold storage.

The stress hormones salicylic acid (SA) and methyl jasmonate (MJ) are also involved in improving natural stress tolerance in crops by enhancing secondary metabolism (Gonzalez-Aguilar et al., 2010; Heredia and Cisneros-Zevallos, 2009). SA is involved throughout plant growth and development and is directly associated with accumulation of protective compounds during systemic acquired resistance (Chen et al., 2006). MJ enhances the defense-signaling pathway, operating through the octadecanoid pathway and eliciting production of secondary metabolites such as phenolic acids (Heredia and Cisneros-Zevallos, 2009; Kim et al., 2006), some of which may act as antioxidants. These secondary metabolites can react with reactive oxygen species (ROS) triggered during stress, and quench ROS activity directly or indirectly. The nutraceutical properties of the fruit can be closely associated to changes in the physico-chemical qualities of the fruit. Thus, the importance of plant-based secondary metabolites as a strong contributor to aesthetic and nutritional properties of fruits and vegetables (Rautiainen et al., 2012; Marnewick et al., 2011).

Nutraceutical properties of fruits and vegetables are of significant interest to consumers, dually as protective compounds for maintaining the fruit quality and for their nutritional benefits. Moreover, Thus, this study aims to explore the effect of SA and MJ in combination with storage at 6 °C could on shelf life and the bioactive content of dragonfruit.

## 2. Materials and Methods

### 2.1 Plant material

Disease free and uniform sized dragonfruit were purchased from a commercial farm in the state of Negeri Sembilan, Malaysia. Freshly harvested fruit that were free from disease and of uniform size and shape were selected, and then washed with 0.05% sodium hypochlorite followed by distilled water and air-dried at ambient temperature.

### 2.2 Treatments of fruit

The fruits were subjected to one of two sets of treatments. The first being the MJ treatment, with fruits subjected to MJ vapour at concentrations of 0.01 mM, 0.1 mM, 0.2 mM and 0.5 mM MJ, along with an untreated control. The fruits were incubated in 45 L airtight storage boxes for 16 h at room temperature, and then allowed to ventilate for 2 h. The second set was the SA treatment, which involved dipping the fruits in SA solution at concentrations of 0.1 mM, 1 mM, 2 mM and 5 mM SA, along with a control dipped in water. After treatment, fruits were packed in cardboard boxes and stored for 21 d at 6 °C, 60-70 % relative humidity, and were assessed at 7 day intervals.

### 2.3 Biochemical quality parameters

Fruit pulp was mixed with distilled water at a ration of 1:10 and homogenized in a blender. Soluble solids content (SSC) was determined using a Palette Digital Refractometer (Model: PR-32α, Atago Co., Ltd. Japan) that was standardised with distilled water. The refractometer was calibrated against sucrose.

The fruit homogenate was titrated against 0.1 N NaOH, using 0.1 % phenolphthalein as an indicator, to determine titratable acidity (Ranganna, 1977). The results were expressed as percentage of citric acid (% citric acid).

$$\%TA = \frac{titre\ value\ x\ vol.made\ up\ x\ Eq\ wt\ of\ citric\ acid\ (64) x\ 100}{sample\ wt\ x\ vol\ sample\ used\ x\ 1000}$$

2.4 Betacyanin content

The betacyanin content and soluble phenolics content of dragonfruit was measured to assess their contribution to the bioactive content of the fruit. Betacyanin content was determined using the spectrophotometric method (Nerd et al., 1999) where fruit pulp was homogenised with 80 % methanol (v/v) and extract was filtered with Whatman no. 1 paper. The extract was mixed with 0.1 M acetate buffer and absorbance measured at 538 nm.

2.5 Soluble phenolics content

Soluble phenolics content was assessed using Folin Ciocalteu spectrophotometric method (Singleton and Rossi, 1965). Fruit pulp was subjected to extraction with 60 % methanol (v/v), at a ration of solute to solvent of 1:10. The extract was reacted with undiluted Folin Ciocalteu reagent (Sigma-Aldrich, USA) at 35 °C for 2 h. Absorbance readings were then measured at 765 nm, and calibrated against a standard curve constructed using freshly prepared gallic acid solution (0 - 1000 µM).

2.6 Antioxidant activity

Total antioxidant activity was determined using the fruit pulp extracted with 60 % methanol (Section 2.5). Two methods were adopted for measuring antioxidant activity: Ferric Reducing Antioxidant Power (FRAP) assay (Benzie and Strain, 1996) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) adapted from the method of Brand-Williams and colleagues (Molyneux, 2004).

Fruit extract was reacted with freshly prepared FRAP reagent and the absorbance was measured at 593 nm after 4 min of incubation at 37 °C, with the FRAP reagent reacted with

60 % methanol as a blank. Results were expressed as mM $kg^{-1}$ Trolox equivalents on a fresh weight basis based on a standard curve prepared beforehand using Trolox (0 – 1000 µM).

For DPPH analysis, fruit extract was mixed with 0.1 M Tris-HCl and 0.3 mM DPPH dissolved in methanol and allowed to react for 20 min at room temperature under dark conditions. Absorbance readings were recorded at 517 nm with a methanol only blank for baseline correction and methanol in DPPH as a control. Results were expressed as mM $kg^{-1}$ Trolox equivalents on a fresh weight basis.

### 2.7 Statistical analysis

The experiment followed a completely randomized design (CRD) with four replicates per treatment, consisting of three fruits per replicate. Analysis of Variance (ANOVA) was used to measure the treatment effect using SAS® 9.1 (SAS Institute Inc., USA), and means were separated using Duncan's Multiple Range Test (DMRT) test at ($P<0.05$).

## 3. Results

### 3.1 Biochemical quality of fruits

SSC and TA were assessed as indicators of the metabolic activity and ripening stage of the fruit. A significant decline ($P < 0.05$) in SSC (Figure 1a) was observed during the storage period. Treatment with 0.5 mM MJ maintained the highest SSC values by the final day of storage. Similarly, TA (Figure 2a) declined during the storage period but at a slower rate, and significantly lower ($P < 0.05$) values were observed for the fruits treated with 0.1 mM MJ. By the final day of storage, all the treatments were at par ($P > 0.05$) with the exception of the 0.01 mM MJ treatment, which exhibited the lowest TA values.

The decline in SSC (Figure 1b) was consistent with the observed ripening pattern for dragonfruit (Figure 1a), however, treatment with 0.1 mM and 1 mM SA maintained

significantly higher ($P < 0.05$) SSC levels throughout the storage period. An initial decline in TA (Figure 4b) ($P < 0.05$) was observed for all treatments including the control, during the storage period. However, TA values of fruits treated with 1 mM SA were significantly higher ($P < 0.05$) on the final day of storage.

### 3.2 Bioactive content of fruits

A gradual increase ($P < 0.05$) in betacyanin content was observed during the storage period (Figure 3a). Fruits treated with 0.5 mM MJ exhibited a peak in betacyanin content by the 14$^{th}$ day of storage, however, the lower doses of MJ (0.01 and 0.1 mM) exhibited the highest levels of betacyanin at the end of the storage period. Meanwhile, no significant effect ($P > 0.05$) of treatment was observed for the soluble phenolics content of the fruits (Figure 4a). Although a decline was observed throughout the storage period, the treatments were at par ($P > 0.05$).

Similarly, an increase ($P < 0.05$) in betacyanin content was observed for the SA treatment (Figure 3b). Moreover, the control maintained higher betacyanin levels during the first two weeks of storage. Lower betacyanin levels were observed for fruits treated with the higher doses of SA (2 and 5 mM) during the first 2 weeks of storage, however, betacyanin levels peaked for these two treatments by the final day of storage. Moreover, soluble phenolic content gradually declined during storage (Figure 4b), with lower values reported for the fruits treated with 2 mM SA.

### 3.3 Antioxidant activity of fruits

A significant effect ($P < 0.05$) of MJ treatment was observed for FRAP antioxidant activity (Figure 5a) throughout the storage period. The antioxidant activity peaked on the 14$^{th}$ day of storage, with the highest peak observed for the higher concentrations of MJ. A 4.2 fold

increase in the initial antioxidant levels were observed for fruits treated with 0.5 mM MJ. By the final day of storage, all the treatments were at par ($P > 0.05$) except for 0.5 mM MJ which exhibited significantly lower levels. DPPH radical scavenging activity (Figure 6a) increased linearly throughout the storage period. The highest level of activity was observed for the control, with the MJ treated fruits displaying significantly lower ($P < 0.05$) activity.

The control (Figure 5b) maintained the highest antioxidant activity during the initial storage period. However, towards the end of the storage period, treatment with SA resulted in significant ($P < 0.05$) increases in the antioxidant activity. The increase in FRAP was positively correlated to the dose of SA applied. This was different to the trend observed for DPPH radical scavenging activity (Figure 6b). The highest activity was reported for the untreated control throughout the storage period, with lower levels of activity observed as the SA dose increased. By the final day of storage, the radical scavenging activity was par ($P > 0.05$) for all the treatments.

## 4. Discussion

The fruits used in this experiment were harvested at maturity, thus at a more advanced ripening stage, which is a common commercial practice for dragonfruit. Gradual decline in the chemical composition of dragonfruit were observed in the experiments. The decline in SSC is attributed to the hydrolysis of insoluble polysaccharides into sugars (Vyas et al., 2015). Organic acids can also be utilized as energy reserves during respiration, accounting for the decline in TA (Valero and Serrano, 2010). SSC and TA both serve as a measure of the changes in the energy reserves that fuel metabolic activities of the plant (Wills et al., 2007). Thus, the gradual decline of SSC and TA can be regarded as an indicator of the metabolic status of the fruit, representing progressive ripening of the fruit. High respiration of the fruit

210 is linked to a relatively short shelf life; thus, the fruits were assessed for only three weeks of
211 storage.

212 MJ promotes both ripening and defence mechanisms of plants through the regulation
213 of gene expression. A positive feedback on the biosynthesis pathway of jasmonic acid has
214 also been reported upon exposure to MJ (Ziosi et al., 2008). However, the results of this
215 experiment indicate that MJ did not influence ripening of dragonfruit. On the other hand, SA
216 treatment did influence ripening, as the higher doses increased the metabolic activity of the
217 fruit, while lower doses decreased the metabolic activity. Reduced metabolism rate and
218 delayed fruit ripening upon exposure to SA has been previously reported in various fruits
219 (Ashgari and Aghdam, 2010). SA has been successfully demonstrated to enhance chilling
220 tolerance in various fruits, such as sponge gourd (Han et al., 2017), plum (Luo et al., 2011)
221 and pomegranate (Sayyari et al., 2011). This protective activity of SA has been primarily
222 associated with enhanced antioxidant activity, increased heat shock proteins and enhanced
223 membrane integrity (Han et al., 2017). However, the protective activity of SA during cold
224 storage has also been attributed to decreasing levels of total phenolic content and antioxidant
225 enzymes in fruits during cold storage (Han et al., 2017; Luo et al., 2011; Sayyari et al., 2011).

226 Previous studies have accounted an increase in antioxidant activity of various fruits
227 upon subjection to MJ (Szymanowska et al., 2015; Wang et al., 2007). Enhanced antioxidant
228 activity, as reported in grapes (Flores et al., 2015) and pomegranates (Sayyari et al., 2011),
229 was associated with enhanced phenolic content and anthocyanin content. While hormonal
230 treatment did not induce a significant effect ($P > 0.05$) on the soluble phenolics content of the
231 fruit, betacyanin content and antioxidant activity was promoted in fruits exposed to MJ.
232 Betacyanin, being the principal bioactive compound in dragonfruit (Muhammad et al., 2014),
233 could have increased during ripening as a defense response towards the induced stresses
234 (Zhang et al., 2003). Fan et al., (2016) reported enhanced antioxidant activity in eggplant,

which was related to delayed senescence of the fruit. Higher doses of MJ are reported to have a more pronounced impact on antioxidant activity (Kim et al., 2006), and this was similarly observed for FRAP activity of the dragonfruit in this study.

While MJ enhanced betacyanin content and antioxidant activity, SA treatment at all doses did not enhance betacyanin content or soluble phenolic content. However, SA treatment did result in an increase in antioxidant activity during the final days of storage. Clearly, another factor is at play in extending the storage period of the fruits treated with lower doses of SA. Studies have related application of SA to increased activity of antioxidant enzymes, such as catalase and superoxide dismutase (Dokhanieh and Aghdam, 2016; Asghari and Aghdam, 2010), which could enhance the antioxidant capacity of the fruit. Nie et al., (2015) reported increased activity of catalase gene expression during cold stress of dragonfruit explants, demonstrating a clear role of this enzyme in cold tolerance. This could be explored further by examining the response of this enzyme in the mature fruit to SA and MJ in combination with the cold stress. Moreover, Asghari and Hasanlooe (2015) reported the importance of positive crosstalk between SA and MJ in enhancing antioxidant activity and natural resistance in strawberry. The interaction of these two stress hormones might also prove beneficial for treating dragonfruit during cold storage.

## 5. Conclusion

An increasing interest in nutritional composition of fruits has been witnessed among consumers lately, however the aesthetic appearance of fruits remains an important parameter for consumer perception of fruit quality. Progressive changes in the physical parameters occur during ripening, accompanied with changes in the chemical and bioactive content affecting both the nutritional and aesthetic properties of the fruit. However, these changes do not essentially follow the same pattern and are largely influenced by external factors such as

cold storage and stress hormones. The effect of the stress hormones SA and MJ on dragonfruit in combination with cold storage have not been previously explored, and these results illustrate the ongoing changes in the fruit metabolic activity and redox homeostasis during cold storage. While lower doses of SA exhibited a role in delaying ripening, it did not enhance the bioactivity of the fruit. Meanwhile, MJ did not have a significant effect on ripening, yet resulted in enhanced betacyanin and antioxidant activity. Future studies could explore the effect of the interaction of these two hormones on dragonfruit.

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