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Effect of Preharvest Treatment Using Jasmonic Acid and Methyl Jasmonate on the Physicochemical Properties and Antioxidant Activities of Red-fleshed Dragon Fruit (*Hylocereus polyrhizus* L.)

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ABSTRACT

This study investigated the effect of exogenous plant growth regulators (PGR), namely jasmonic acid (JA) and methyl jasmonate (MeJA), on the physicochemical properties of flesh and peels of red-fleshed dragon fruit (*Hylocereus polyrhizus*). The fruit was sprayed with 100 and 1,000 ppm of JA and MeJA at 15 and 22 days of anthesis and harvested after 35 days. Then, the flesh and peels were analyzed for total soluble solids (TSS), total betacyanins, betanin, total phenolics (TP), total flavonoids (TF), and color characteristics. The fruit peels contained significantly higher (p<0.05) TP and antioxidant activities compared to flesh. No significant difference was detected between the variables in the peels, except for significantly higher (p<0.05) of total betacyanins (~295.6 and ~299.9 mg/100 g) and TP (~614.1 and 566.1 mg GAE/100 g) were recorded in control and 100 ppm MeJA, respectively. In the flesh, 1,000 ppm MeJA-treated fruit possessed the highest total betacyanins (~139.2 mg/100 g), betanin (~356.0 mg/g), TP (~244.9 mg GAE/100 g), TF (~329.0 mg CE/100 g), Trolox equivalent antioxidant capacity (TEAC) (63.2 μmol TE/g) and reducing power (~21.5 μmol TE/g). Overall, 1,000 ppm MeJA was more effective in enhancing the accumulation of bioactive compounds and antioxidant activities in the flesh of red-fleshed dragon fruit compared to other PGR treatments.

Keywords: Betacyanins, fruit preservation, pitaya, plant growth regulator, plant hormone, shelf life extension, sustainable agriculture

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INTRODUCTION

Preference and consumption of fruit depend on several aspects, including the fruit's visual cues and taste and nutritional benefits. The red-fleshed dragon fruit (*Hylocereus polyrhizus*) has been recognized for its attractiveness, unique shape, intense red colors, juiciness, sweetness, and pleasant taste. It is a native of Southern Mexico and Central America (Abirami et al., 2021). Dragon fruit has become popular in Asia, and cultivation is expanding in countries such as Vietnam, Thailand, Taiwan, the Philippines, and Malaysia (Hossain et al., 2021). Three main components in dragon fruit include the pulp (47.40%–73.76%), peel (36.70%–37.60%), and seed (2.70%–14.67%) with an average weight of approximately 300–350 g. It can be consumed as a dessert or salad or used to produce various products such as juices, jams, candies, and ice creams. Other than flesh, the fruit peels are enriched with beneficial polyphenols, especially betacyanins, that could be further processed into valuable products such as natural food colorants (Jalgaonkar et al., 2022).

Notably, the red-fleshed's nutrient composition, minerals, and vitamins vary by cultivar, agricultural region and climate, plant feeding and nutrition, ripening stage, harvesting duration, and storage conditions (Attar et al., 2022). The bioactive components, including vitamin C, phenolic acids, betalains, and flavonoids, are ubiquitously found in the fruit to serve a variety of protective functions such as antioxidant, antimicrobial, anticancer, and antidiabetic activities (Nishikito et al., 2023). These molecules have become increasingly important in the human diet and remarkably increased the market of red-fleshed dragon fruit domestically and internationally (Jalgaonkar et al., 2022).

Specific metabolic pathways have created natural pigments and can be classified into several large groups, such as carotenoids, betalains, chlorophyll, and anthocyanins. Betalains are recognized as a group of water-soluble nitrogen-containing pigments. They are also known as chromo-alkaloids due to the presence of nitrogen in the basic structural components (Ren et al., 2017). Betalains can be further classified into two primary structural subgroups, betacyanins and betaxanthins, which are responsible for the reddish-violet and yellowish-orange colors, respectively (Miguel, 2018).

Betacyanins abundant in red-fleshed dragon fruit, identified as betanin, isobetanin, philocactin, and hilocerenin (Cheok et al., 2022). These compounds are important in providing an intense red color in the fruit peels and flesh. Notably, betacyanin stability is affected by various factors, including temperature, pH, water activity, light intensity, oxygen, and antioxidants (Calva-Estrada et al., 2022). These factors might lead to the deformation or degradation of betacyanin compounds. Given the brilliant red color of the fruit, it can fulfill other functions, such as attracting pollinators for pollination, seed dispersal, photosynthesis, and protection against biotic and abiotic stress (Sadowska-Bartosz & Bartosz, 2021).

Recently, the study of betacyanins in the red-fleshed dragon fruit has attracted much attention due to current evidence about the positive influences on the human body, as it also acts as an antioxidant (Paśko, Galanty, Zagrodzki, Ku, et al., 2021).

Therefore, the increased demand for tropical fruit, including dragon fruit, results in the agroindustry continuing to expand the production of high-quality fruit. Furthermore, new approaches using pre-treatment to improve the quality of fruit crops are continuously being developed. One of the pre-treatments developed involves gene regulation modification by applying plant growth regulators (PGR) on fruit crops (Ordoñez Trejo et al., 2023). The application of PGR is widely used in modern agriculture to enhance fruit crops' physicochemical properties and desirable bioactive compounds, improving the market's desirable characteristics. PGR are chemical compounds that are synthesized artificially to mimic and perform the same function as naturally occurring plant hormones, including auxins, cytokines, gibberellins, acetic acid, ethylene, salicylic acid, and the jasmonates group (Mukherjee et al., 2022).

PGR could effectively improve the characteristics and accumulation of bioactive compounds in fruit crops (Ordoñez Trejo et al., 2023). However, the effectiveness of PGR performance may also be affected by factors such as application method, time of application, concentration, crop stage, plant species, and the environmental conditions in which plants are cultivated (Khan et al., 2020). Moreover, PGR can be applied to plants through a variety of methods. The most common include foliar application, soaking, pre-plant seeding, and injection.

Jasmonic acid (JA) and its derivatives were called 'jasmonates.' Naturally, jasmonates are lipid-derived compounds synthesized via the octadecanoid pathway (Figure 1). Wasternack and Strnad (2018) reported that the initial step of JA synthesis occurs in the membrane chloroplast, where α-linolenic acid from membrane lipids is released. Moreover, the synthesis of JA in the chloroplast is regulated by enzymes such as lipoxygenase (LOX), allene oxide synthase (AOS), and allene oxide cyclase (AOC). These enzymes are important in various ways; LOX contributes to ripening, aroma, and flavor development, AOS can act as antioxidants and defense mechanisms against pathogens and insects, and AOC is linked to environmental stress responses, fruit ripening and senescence. Also, these enzymes are responsible for forming oxophytodienoic acid (OPDA). Hence, the ultimate form of JA could be produced when OPDA is transported into peroxisomes and oxidized by the process of β-oxidation. In summary, after the formation of OPDA, the jasmonic acid biosynthetic pathway continues through a series of enzymatic conversions to produce active jasmonic acid. This molecule then plays a crucial role in regulating various processes contributing to fruit quality, including ripening, aroma, flavor, defense responses, and stress tolerance. In addition,

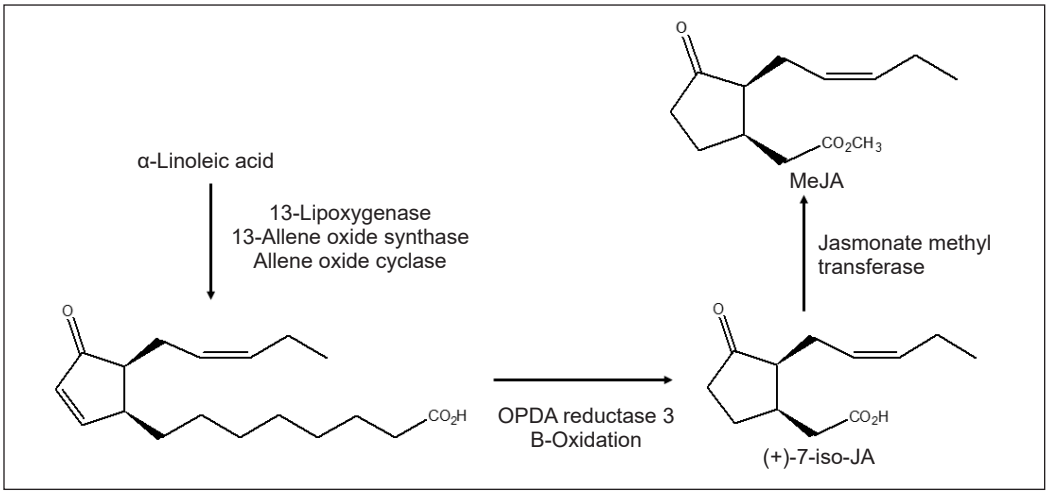

Figure 1. Structure and biosynthesis of jasmonates
Note. OPDA = 12-oxophytodienoic acid; JA = Jasmonic acid; MeJA = Methyl jasmonate (Source: Taki-Nakano et al., 2014)

methyl jasmonate (MeJA) could be formed if the JA produced is further altered in the cytoplasm by jasmonate methyl transferase (Ruan et al., 2019).

The information about exogenous JA and MeJA regulating the bioactive compounds in red-fleshed dragon fruit is limited. The research problem in this study revolves around the limited knowledge regarding the impact of exogenous JA and MeJA on the regulation of bioactive compounds in red-fleshed dragon fruit, the unexplored effects of preharvest treatment with varying concentrations of JA and MeJA on the physicochemical properties and antioxidant activity, as well as inconsistency in the quality of both the flesh and peels of this fruit. Therefore, this study aims to investigate the effect of preharvest treatment using 100 and 1,000 ppm of JA and MeJA on the physicochemical properties, bioactive compounds, antioxidant activity, and color improvement of the flesh and peels of red-fleshed dragon fruit. In addition, the Pearson correlation, general linear model (GLM), and principal component analysis (PCA) were also performed to correlate between the variables studied in the fruit flesh and peels. Overall, preharvest treatment with a higher concentration of JA and MeJA at 1,000 ppm was hypothesized to significantly influence the variables tested on the flesh and peels of red-fleshed dragon fruit compared to 100 ppm.

MATERIALS AND METHODS

Chemicals

The solvent and reagent used were of analytical and high-performance liquid chromatography (HPLC) grade. Folin–Ciocalteu's phenol reagent and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) were obtained from Merck (Germany). Betanin, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and 6-hydroxy-2,5,7,8-

tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (USA). Ethanol, trifluoroacetic acid (TFA) 98%, and acetonitrile were purchased from Fisher Scientific (United Kingdom).

Agronomic Practices of the Plant

The agronomic practices of the red-dragon fruit plant were carried out at the Multi Rich Pitaya farm located in Sepang, Selangor, Malaysia (GPS: 2.805723, 101.7407). The planting distance was 3 m × 3 m using a single post system. The age of the plant was six years. Five kg of organic manure was applied to each plant once a year. In contrast, chemical fertilizer containing nitrogen (N), phosphorus (P), and potassium (K) was applied at the ratio of 2:10:10 (v:v:v) for every month. Irrigation and pest or disease management are interconnected aspects of modern agriculture. Hence, proper irrigation practices, including water management, can help prevent the development and spread of pests and diseases. Also, integrating irrigation management into a holistic pest and disease management strategy is crucial for maintaining crop health and maximizing yields while minimizing the use of chemical controls.

Phytohormone Preharvest Treatments

The application of PGR was carried out according to the method by H. Wang et al. (2019) with slight modifications. The fruit was sprayed before 9 a.m. (28°C, 63% relative humidity [RH]) with 100 and 1,000 ppm of JA and MeJA after 15 and 22 d of anthesis. The spraying volume was around 8-10 ml, covering the entire fruit. The sprayer was designed to deliver smaller droplets (<150 μm) to control the drifting of treatments. At least 10 flowers were treated in each treatment, where control samples were the red-fleshed dragon fruit flowers without any treatment. The fruit was then harvested after 35 d of anthesis. The criteria for harvesting the fruit involve a vibrant red skin color and a uniform shape, with each fruit weighing around 300 g. The harvested fruit were packed in the polystyrene box (55 length [L] × 42 width [W] × 30 height [H] cm; ±70 L) and then transported in air-conditioned transportation (25°C, 27% RH) to the laboratory and kept at 4°C until further analysis.

Preparation of Red-fleshed Dragon Fruit Extract

Flesh. Preliminary experiments were conducted to determine the conditions favoring better betacyanins extraction. The extraction methods were performed according to Naderi et al. (2012) with slight modifications, whereas the extraction of betacyanins from flesh used a 1:1 ratio of flesh/ethanol (w/v). A 100 g of the mashed flesh fruit was typically macerated with 100 ml of 50% aqueous ethanol for 15 min under cooling conditions (4°C).

Peel. Fruit peels were separated from the flesh and dried for 48 hr. Freeze-dried peels were ground and passed through 20 mesh sieves. Then, 4 g of peel powder was added to 100 ml of 50% ethanol to be macerated for 15 min under cooling conditions (4°C).

The homogenized sample was centrifuged at 769 × *g* and 4°C for 15 min, followed by vacuum filtration using Whatman No.1 filter paper (Sigma-Aldrich, USA). The extraction for both flesh and peels was conducted in triplicate. Then, the supernatants were kept at -20°C for further analysis.

Determination of Total Soluble Solids (TSS)

TSS of red-fleshed dragon fruit flesh was determined using an ATAGO digital handheld pocket refractometer PAL-08S (Japan). The values in °Brix were taken in triplicate measurements.

Determination of Total Betacyanins

Total betacyanins were quantified according to the Naderi et al. (2012) method with slight modifications, whereby the solvent used was 50% ethanol instead of deionized water. The extracted samples were diluted 20-fold with ethanol and kept in the dark for 20 min for equilibrium. The following equation describes the quantification of total betacyanins:

$$\text{Total betacyanins (mg/L)} = \frac{A \times DF \times MW \times 1{,}000}{\varepsilon \times L} \quad (1)$$

where, A = absorption at 540 nm, F = dilution factor, MW = 550 g/mol (molecular weight of betanin), ε = 60,000 L/mol·cm (molar extinction coefficient of betanin in ethanol), and L = 1 cm (pathlength of the cuvette). Total betacyanins were calculated and expressed in mg per gram (mg/g).

$$\text{Total betacyanins (mg/g)} = \frac{\text{Total betacyanins } (\frac{\text{mg}}{\text{L}}) \times (L)}{\text{Sample (g)}} \quad (2)$$

Quantification of Betanin Using HPLC

Betanin was determined based on the method of Naderi et al. (2012) with slight modifications. The mobile phase contained a mixture of 90% solvent A (0.5% aqueous TFA) and 10% solvent B (acetonitrile). Briefly, a Purospher STAR RP18 end-capped column (250 mm × 4.6 mm i.d., particle size of 5 µm, Merck, Germany) in liquid chromatographic apparatus (Waters Corp., USA), equipped with a Waters 2478 two-channel UV detector was used to separate and quantify the betanin at 540 nm. The flow rate was 1 ml/min, and the injection volume was 10 µl for 15 min of analysis. The calibration curve for betanin was plotted in the range of 10.0–2.0 mg/ml.

Determination of Total Phenolics (TP)

TP was determined according to the Folin Ciocalteu's reagent as described by Ramli et al. (2014) with slight modifications. The TP was quantified using the standard curve of gallic acid (0.25–0.05 mg/ml in water), which was expressed in mg gallic acid equivalent (GAE) per 100 g (mg GAE/100 g).

Determination of Total Flavonoids (TF)

The TF in red-fleshed dragon fruit was analyzed using the method by Senevirathna

et al. (2021). It was calculated using the standard curve of catechin (300–50 mg/L), which was expressed in mg of catechin equivalent per 100 g (mg CE/100 g).

Determination of Trolox Equivalent Antioxidant Capacity (TEAC)

The antioxidant capacity of flesh and peels was carried out according to Nawawi et al. (2023) with slight modifications in the solvent used, concentration of DPPH solution and the ratio of sample to DPPH solution. An amount of 0–2,000 µM of Trolox solution was measured using a spectrophotometer at 517 nm. The antioxidant capacity was expressed as µmol of Trolox equivalents (TE) per gram of the samples (µmol TE/g).

Determination of Ferric-reducing Antioxidant Power (FRAP)

The ferric-reducing antioxidant power (FRAP) was analyzed according to Azman et al. (2022). The concentration of Trolox solution was 0 – 1,500 µM, and reducing power was expressed as µmol of TE per gram (µmol TE/g).

Determination of Color

The color of the flesh and peels of red-fleshed dragon fruit were measured by using a colorimeter (CR-410, Minolta, Japan) based on three coordinates units: L^* (lightness/darkness), a^* (redness/greenness), and b^* (yellowness/blueness), which white tiles are used to calibrate the instrument.

Chroma (C) is the quantitative attribute that describes the intensity of a color, while hue (h°) provides a qualitative attribute of color characterized as reddish, greenish, yellowish, and bluish, respectively. The chroma and hue angle were calculated using the equations below (Wrolstad & Smith, 2017):

$$(C) = [(a^*)^2 + (b^*)^2]^{1/2} \quad (3)$$

$$(h°) = \text{Arctan}\ (b^*/a^*) \quad (4)$$

Statistical Analysis

One-way analysis of variance (ANOVA) was performed in all statistical analyses. In this study, a significant level of 95% (p<0.05) was applied in Tukey's multiple range tests. The correlations between tested parameters were also evaluated using the linear Pearson correlation, GLM and PCA. The statistical analysis was performed using the Minitab V.19 software (Minitab Inc., USA).

RESULTS AND DISCUSSION

According to the preliminary test, freeze-drying is not an effective option to dry the flesh of red-fleshed dragon fruit due to the fruit flesh being high in sugar such as glucose, fructose, and some oligosaccharide (Huang et al., 2021), which produces a sticky powder when freeze-dried. Therefore, in this study, the fresh weight (FW) results were converted into dry weight (DW) based on the moisture content of the flesh (84.0 ± 0.2%). Also, in the preliminary test, the extraction efficiency using methanol or ethanol has been compared. Ethanol was more efficient in extracting bioactive compounds in red-fleshed dragon fruit flesh

and peels due to the polarity of betacyanins. Halimfanezi and Asra (2020) state that betacyanins are more hydrophilic and easier to dissolve in ethanol.

Total Soluble Solids (TSS)

TSS was assessed as an indicator of the sweetness of the fruit. The effects of MeJA and JA foliar treatment on the TSS of fruit flesh are shown in Table 1. After the application of PGR at different concentrations, 100 ppm MeJA, 100 ppm JA, 1,000 ppm MeJA and 1,000 ppm JA, TSS values in the fruit flesh demonstrated a significant (p<0.05) increase (13.13–13.97 ºBrix) compared to the control sample (~12.40 ºBrix). Moreover, statistical analysis verified that only PGR types and their concentrations significantly influenced (p<0.05) the TSS values, while the interaction between these variables showed no significant differences (Table 2).

Similar results were reported in previous studies about the effect of preharvest treatments on the TSS of different fruit species. Due to external preharvest treatment, the TSS value in blackberry (Hull Thornless) increased to 35% after the treatment using 0.1 mM MeJA (S. Y. Wang et al., 2008). On the other hand, TSS in peaches increased by 8% after being treated with 0.1 mmol/L MeJA at 5°C during 14 d of storage (Meng et al., 2009). An increase in TSS value might be due to the conversion of polysaccharides into sugars in the presence of organic acids (Batista-Silva et al., 2018; Gupta et al., 2023).

Total Betacyanins

As reported previously, red-fleshed dragon fruit is a good source of betacyanins, as it is responsible for the red-violet color of the fruit. In this study, the MeJA and JA at different concentrations (100 and 1,000 ppm) were used to provoke the possible stimulation of betacyanins in the flesh and peels, as shown in Table . GLM analysis in Table 2 showed the significant influence of PGR concentrations and the interaction between the PGR types and concentrations (p<0.05). Higher concentrations of PGR efficiently elevated the production of

Table 1
Total soluble solids (TSS), total betacyanins, and betanin in the flesh and peels of untreated (control) and treated red-fleshed dragon fruit with plant growth regulators (PGR) at different concentrations

Treatments (ppm)	TSS (°Brix) (Flesh)	Total betacyanins (mg/100 g)		Betanin (mg/g)	
		Flesh	Peels	Flesh	Peels
Control	12.4 ± 0.1^{c}	107.2 ± 1.0^{Bd}	295.6 ± 1.9^{Aa}	244.6 ± 1.7^{Bd}	734.7 ± 40.8^{Aa}
100 MeJA	13.9 ± 0.1^{a}	109.5 ± 1.6^{Bcd}	299.9 ± 1.6^{Aa}	318.1 ± 1.1^{Bc}	779.87 ± 103.4^{Aa}
100 JA	14.0 ± 0.1^{a}	116.0 ± 2.4^{Bc}	163.4 ± 1.0^{Ad}	328.8 ± 15.7^{Abc}	363.8 ± 58.6^{Ab}
1000 MeJA	13.1 ± 0.1^{b}	139.2 ± 0.1^{Ba}	251.9 ± 6.8^{Ab}	440.4 ± 7.3^{Ba}	583.9 ± 27.9^{Aab}
1000 JA	13.9 ± 0.1^{a}	127.0 ± 3.1^{Bb}	236.5 ± 0.1^{Ac}	356.0 ± 2.2^{Bb}	722.7 ± 35.7^{Aa}

Note. JA = Jasmonic acid; MeJA = Methyl jasmonate. Values with the same letter $^{a, b,}$ and ^{c} in each row are not significantly different (p>0.05). Values with the same letter ^{A} and ^{B} in each column are not significantly different (p>0.05)

Table 2
Summary of the estimation results of the general linear model (GLM)

Analysis of variance	Parameters (*p*-values)		
	Types of PGR	Concentration of PGR	Types of PGR × Concentration of PGR
Flesh			
Total Soluble Solids	0.048	0.000	0.074
Total Betacyanins	0.125	0.000	0.001
Betanin	0.001	0.000	0.000
TP	0.339	0.001	0.014
TF	0.020	0.000	0.000
TEAC	0.000	0.000	0.002
Reducing Power	0.003	0.000	0.000
*L**	0.511	0.055	0.844
*a**	0.522	0.009	0.710
*b**	0.445	0.000	0.838
Peels			
Total Betacyanins	0.000	0.000	0.000
Betanin	0.031	0.019	0.001
TP	0.010	0.000	0.000
TF	0.000	0.132	0.000
TEAC	0.364	0.016	0.743
Reducing Power	0.674	0.303	0.890
*L**	0.001	0.966	0.046
*a**	0.010	0.453	0.001
*b**	0.990	0.156	0.106

Note. TP = Total phenolics; TF = Total flavonoids; TEAC = Trolox equivalent antioxidant capacity. Parameters with $p>0.05$ are not significantly different

betacyanins in the fruit flesh. Among the concentrations tested, the most effective ($p<0.05$) was 1,000 ppm MeJA with total betacyanins of ~139.2 mg/100 g. The result also showed that the application of MeJA is more effective than JA in accumulating betacyanins in the flesh. It might be related to the physical properties of the MeJA that are higher in volatility and hydrophobicity than JA, which make it significantly easier to pass through the plant stomata and reach the inside of the cytoplasm (Li et al., 2018).

A similar finding was reported by Mustafa et al. (2018). In contrast, applying 0.1 mM MeJA on red-fleshed dragon fruit during postharvest treatment increased the accumulation of betacyanins in the fruit, ranging from 40–50 g/kg at 6°C during 14 d of storage. The resulting accumulation of bioactive compounds such as betalain might be due to activating the phenylpropanoid pathway that MeJA and JA regulate. Betalain is a derivation of tyrosine. Thus, phenylpropanoid biosynthesis was acknowledged to be the upstream pathway

of the tyrosine pathway (Yadav et al., 2020). In peaches, a modulating initial effect of PGR was reported similarly, whereas MeJA stimulated the expression levels of anthocyanin-associated genes that resulted in anthocyanin accumulation in the peach fruit (Wei et al., 2017). Therefore, this study demonstrates that PGR could modify the pathway associated with bioactive compounds to increase their concentration in fruit.

In contrast to the flesh, the peels in fruit treated with 100 ppm MeJA and control samples exhibited the highest total betacyanins, ~299.9 and ~295.6 mg/100 g, respectively (Table 1). Statistical analysis also detected a significant influence of the types of PGR, concentrations used and the interaction between the types of PGR and concentrations on the total betacyanins in fruit peels (Table 2).

Overall, total betacyanins in peels (~163.4 – ~299.9 mg/100 g) were higher compared to the flesh (~107.2 – ~139.2 mg/100 g). This result was in agreement with the study by Khoo et al. (2022), where red-fleshed dragon fruit peel had higher betacyanins (~35.12 mg/g FW) than in the flesh (~0.15 mg/g FW). During the extraction process, the broken seeds can also contribute to the degradation of betacyanins in the flesh (Naderi et al., 2012).

Preharvest and postharvest treatments can have distinct physiological effects on fruit, leading to different outcomes in terms of quality and responses. Preharvest treatments

Table 3
Pearson correlation between analysis measured in the flesh and peels of red-fleshed dragon fruit

Analysis 1	Analysis 2	Correlation	*p*-value
Flesh			
Betanin	Total betacyanins	0.923	0.000
TP	Total betacyanins	0.899	0.000
Reducing power	Total betacyanins	0.953	0.000
TF	Total betacyanins	0.935	0.000
TP	Betanin	0.837	0.003
Scavenging activity	Betanin	0.733	0.016
Reducing power	Betanin	0.965	0.000
TF	Betanin	0.829	0.003
Reducing power	TP	0.917	0.000
TF	TP	0.916	0.000
Reducing power	Scavenging activity	0.657	0.039
TF	Reducing power	0.909	0.000
Peels			
Betanin	Total betacyanins	0.874	0.001
TF	Total betacyanins	0.910	0.000
TF	Betanin	0.748	0.013

Note. TP = Total phenolics; TF = Total flavonoids. Analysis with $p<0.05$ is significantly different

involve applying various substances (such as chemicals or growth regulators) to plants before harvesting. These treatments can affect the plant's physiological processes during its growth and development. They can influence nutrient uptake, hormone regulation, and stress responses. On the other hand, postharvest treatments occur after the fruit is harvested and aim to maintain or improve its quality during storage, transportation, and sale. These treatments can include control of ripening, prevent spoilage, and enhance shelf life. It is important to note that while preharvest and postharvest treatments can have varying effects, they are interconnected. Preharvest conditions can set the stage for how fruit responds to postharvest treatments. Additionally, understanding the interactions between these treatments and the JA pathway requires careful consideration of the specific plant species, environmental conditions, and physiological processes involved (Baek et al., 2021).

Betanin

In this study, betanin was the major betacyanin found in the flesh and peels of red-fleshed dragon fruit (Figure 2), which agrees with the data obtained by Naderi et al. (2012). According to Table 1, a significantly higher (p<0.05) concentration of betanin in the flesh (~440.4 mg/g) was recorded in the fruit treated with 1,000 ppm MeJA. However, unlike the peel, no significant difference was observed between all treatments, including the control sample. The Pearson correlation in Table 3 indicated a strong correlation (p<0.05) between the total betacyanins and betanin in the fruit flesh (r = 0.923) and peels (r = 0.874). Also, GLM analysis showed that the types of PGR, concentrations used, and interactions between the types of PGR and their concentrations significantly affected (p<0.05) the betanin in the flesh and peels (Table 2).

On the other hand, Naderi et al. (2012) also quantified the other types of betacyanins such as isobetanin, phyllocactin, isophyllocactin, and hylocerenin. However, only betanin was quantified in this study due to the limitation of the standard. In addition, other studies also reported the findings of anthocyanins in red-fleshed dragon fruit, including cyanidin 3-*O*-glucoside, delphinidin 3-*O*-glucoside, and pelargonidin 3-*O*-glucoside in the average concentration of 12.67, 0.82, and 1.76 mg/100 g DW, respectively (Saenjum et al., 2021). Notably, none of these anthocyanins were detected in this study samples during the preliminary test. It might be due to the extraction process using an acidified hydroalcoholic solution at pH 2 by Saenjum et al. (2021), compared to 50% ethanol in this study.

Total Phenolics (TP) and Total Flavonoids (TF)

Red dragon fruit is known for its phenolic and flavonoid compounds with antioxidant properties. Chen et al. (2021) found that 80 different phenolic compounds were tentatively characterized in dragon fruit, including 25 phenolic acids and 38 flavonoids. The total phenolics and flavonoids in red dragon fruit can vary

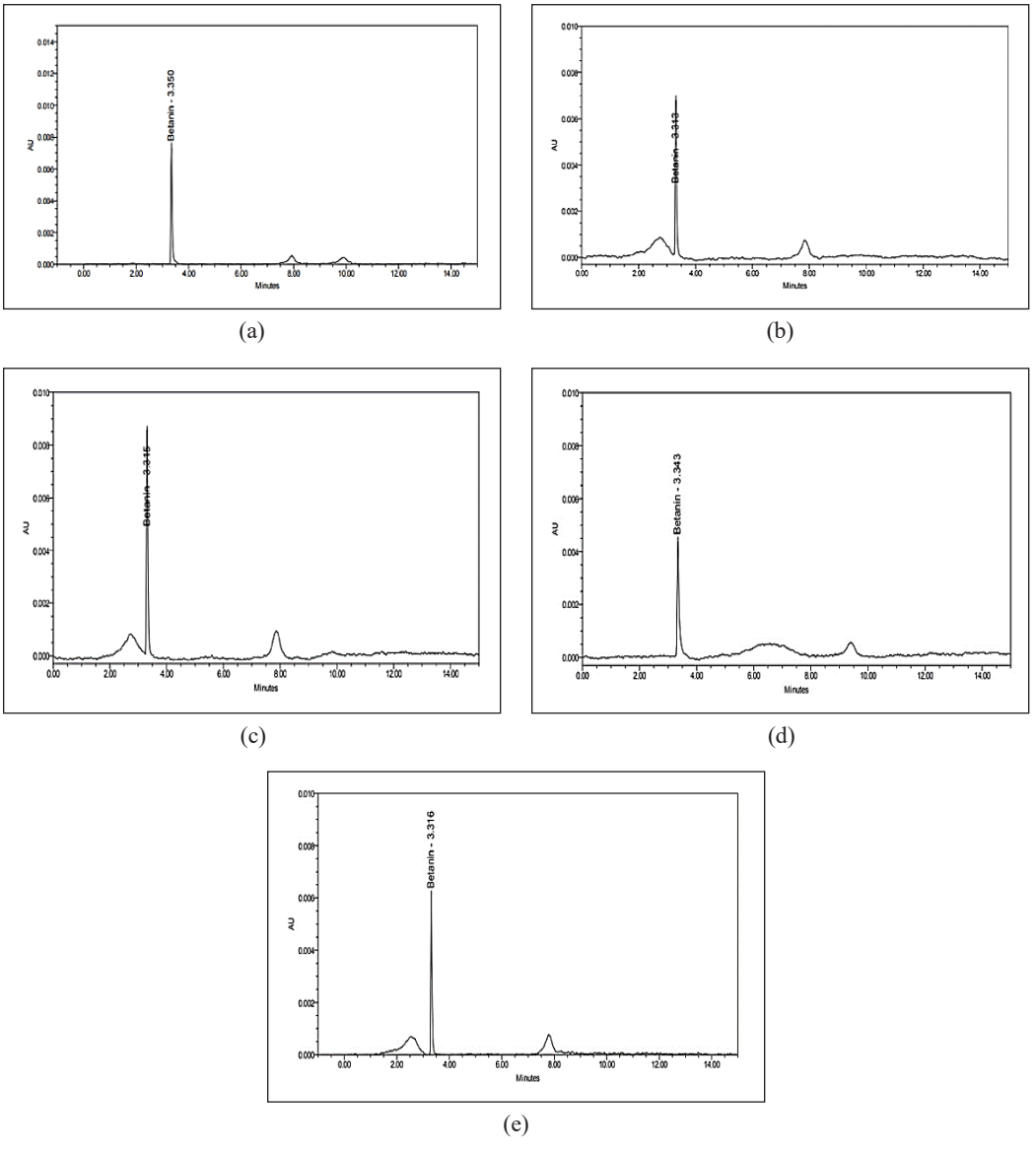

Figure 2. Typical high-performance liquid chromatography (HPLC) chromatogram of (a) standard betanin and betanin detected in red-fleshed dragon fruit flesh of (b) 1,000 ppm JA, (c) 1,000 ppm MeJA and fruit peels of (d) 1,000 ppm JA, and (e) 1,000 ppm MeJA at 540 nm
Note. JA = Jasmonic acid; MeJA = Methyl jasmonate

depending on geographical origin and extraction methods (Paśko, Galanty, Zagrodzki, Luksirikul, et al., 2021).

This study reported the effect of PGR preharvest treatment on the TP and TF of red-fleshed dragon fruit flesh and peels (Table 4). The fruit treated with 1,000 ppm MeJA possessed a significantly higher (p<0.05) TP (~244.9 mg GAE/100 g) and TF values (~329.0 mg GAE/100 g)

Table 4
Total phenolics (TP), total flavonoids (TF), Trolox equivalents antioxidant capacity (TEAC), and reducing power in the flesh and peels of untreated (control) and treated red-fleshed dragon fruit with plant growth regulators (PGR) at different concentrations

Treatments (ppm)	TP		TF		TEAC		Reducing power	
	Flesh	Peels	Flesh	Peels	Flesh	Peels	Flesh	Peels
	mg GAE/100 g		mg CE/100 g		µmol TE/g		µmol TE/g	
Control	208.3 ± 2.0^{Bb}	614.1 ± 5.7^{Aa}	225.5 ± 6.1^{Bbc}	774.7 ± 14.3^{Ab}	33.2 ± 0.2^{Bc}	90.3 ± 13.3^{Aa}	15.7 ± 0.2^{Bd}	44.4 ± 4.5^{Aa}
100 MeJA	202.1 ± 5.1^{Bb}	566.1 ± 4.3^{Aa}	201.8 ± 9.2^{Bc}	846.6 ± 37.5^{Aa}	60.4 ± 5.3^{Ba}	75.4 ± 4.9^{Aa}	16.9 ± 0.2^{Bc}	40.8 ± 11.9^{Aa}
100 JA	213.8 ± 20.3^{Bb}	458.1 ± 21.9^{Ab}	234.2 ± 0.1^{Bb}	640.3 ± 24.8^{Ac}	44.2 ± 1.0^{Bb}	66.4 ± 1.9^{Aa}	18.0 ± 0.5^{Bb}	36.4 ± 3.3^{Aa}
1000 MeJA	244.9 ± 3.3^{Ba}	300.1 ± 26.8^{Ac}	329.0 ± 6.1^{Ba}	787.2 ± 21.7^{Aab}	63.2 ± 1.4^{Ba}	70.7 ± 0.1^{Aa}	21.5 ± 0.1^{Ba}	36.9 ± 0.4^{Aa}
1000 JA	220.4 ± 6.5^{Bab}	486.5 ± 26.7^{Ab}	255.7 ± 12.2^{Bb}	724.7 ± 28.1^{Ab}	43.8 ± 0.12^{Bb}	66.0 ± 3.3^{Ab}	18.3 ± 0.0^{Bb}	36.1 ± 9.0^{Aa}

Note. JA = Jasmonic acid; MeJA = Methyl jasmonate. Values with the same letter $^{a, b,}$ and ^{c} in each row are not significantly different (p>0.05). Values with the same letter ^{A} and ^{B} in each column are not significantly different (p>0.05)

compared to other parameters, including control samples. According to the statistical analysis, the concentrations of PGR used and the interactions between the types of PGR and their concentrations significantly influenced (p<0.05) the TP values in the fruit flesh (Table 2). Also, in the flesh, there were strong correlations (p<0.05) between TP and total betacyanins (r = 0.899) and betanin (r = 0.837). Moreover, strong correlations (p<0.05) were detected between TF and total betacyanins (r = 0.935) and betanin (r = 0.829) (Table 3).

In the case of fruit peels, control and 100 ppm MeJA demonstrated a significantly higher TP (p<0.05), ~614.1 mg GAE/100 g, and ~566.1 mg GAE/100 g, respectively, compared to other samples. On the other hand, higher TF values were recorded in 100 ppm MeJA (~846.6 mg GAE/100 g) and 1,000 ppm MeJA (~787.2 mg GAE/100 g). Overall, TF in the flesh and TP in the peels were significantly influenced (p<0.05) by the types of PGR, their concentrations and the interactions between the PGR types and their concentrations. In the peels, TF was strongly correlated (p<0.05) to total betacyanins (r = 0.910) and betanin (r = 0.748).

The effect of PGR treatment on the increment of the TP is also reported in other cultivar fruits. In the study by Ghasemzadeh et al. (2016), a significant increase of 64% in TP and 234% in TF value was reported in sweet potato root after being subjected to the treatment using 100 µM MeJA. Hu et al. (2022) also claimed that applying JAs can activate a signal molecule that could trigger the plant defense systems and increase phenolic synthesis.

Antioxidant Activities (TEAC and Reducing Power)

Two or more assays are commonly used to determine the antioxidant capacity of the plant extract. Various assays are used to determine antioxidant activity because they could exert their effect in various mechanisms, such as scavenging radicals, sequestering transition metal ions and decomposing hydrogen peroxide (Chaves et al., 2020). Hence, the chosen best-suited method should consider the function to be evaluated. In this study, the TEAC and FRAP assays were used to evaluate the antioxidant capacities of the red-fleshed dragon fruit. In general, the TEAC assay was used to measure the antioxidant capacity, while the FRAP assay was used to determine the reducing power of samples (Chen et al., 2021).

As shown in Table 4, the TEAC values were highest in the fruit flesh after 100 and 1,000 ppm MeJA treatments, resulting in ~60.4 and ~63.2 µmol TE/g, respectively. The highest reducing power in the flesh was detected in the fruit treated with 1,000 ppm MeJA (~21.5 µmol TE/g). According to the GLM analysis, the types of PGR, concentrations used, and their interactions significantly influenced (p<0.05) the TEAC and reduced power in the fruit flesh.

The stronger antioxidant activity in red-fleshed dragon fruit is likely due to the

Table 5
Color characteristics in the flesh and peel of untreated (control) and treated red-fleshed dragon fruit with plant growth regulators (PGR) at different concentrations, as measured by colorimeter

Flesh						
Treatments (ppm)	*L**	*a**	*b**	C	h°	Visualization
Control	29.85 ± 0.69^{a}	36.92 ± 1.61^{a}	-2.10 ± 0.28^{c}	36.98 ± 1.61^{a}	-0.06 ± 0.01^{a}	
100 MeJA	30.09 ± 0.76^{a}	36.30 ± 1.19^{a}	-1.71 ± 0.54^{bc}	36.34 ± 1.19^{a}	-0.05 ± 0.01^{a}	
100 JA	30.22 ± 0.60^{a}	37.14 ± 0.83^{a}	-1.84 ± 0.56^{ab}	37.18 ± 0.82^{a}	-0.05 ± 0.02^{a}	
1,000 MeJA	29.34 ± 0.83^{a}	35.30 ± 1.83^{ab}	-0.43 ± 0.51^{a}	35.30 ± 1.82^{a}	-0.01 ± 0.01^{a}	
1,000 JA	29.64 ± 0.93^{a}	35.34 ± 1.93^{ab}	-0.63 ± 0.65^{a}	35.35 ± 1.94^{a}	-0.02 ± 0.02^{a}	
Peels						
Treatments (ppm)	*L**	*a**	*b**	C	h°	Visualization
Control	40.72 ± 3.07^{ab}	41.12 ± 3.67^{a}	8.41 ± 1.40^{a}	42.02 ± 3.35^{a}	0.21 ± 0.05^{a}	
100 MeJA	39.67 ± 1.51^{ab}	40.38 ± 2.23^{ab}	7.54 ± 0.73^{a}	41.09 ± 2.13^{ab}	0.19 ± 0.02^{a}	
100 JA	42.11 ± 2.64^{ab}	40.09 ± 2.39^{ab}	8.17 ± 1.47^{a}	40.95 ± 2.23^{ab}	0.20 ± 0.04^{a}	
1,000 MeJA	39.25 ± 2.59^{b}	37.76 ± 3.51^{b}	8.35 ± 0.71^{a}	38.68 ± 3.42^{b}	0.22 ± 0.03^{a}	
1,000 JA	42.22 ± 2.14^{a}	42.94 ± 1.50^{a}	7.73 ± 0.70^{a}	43.64 ± 1.49^{a}	0.18 ± 0.02^{a}	

Note. JA = Jasmonic acid; MeJA = Methyl jasmonate. Visualization = Conversion of *L**, *a**, and *b** values to RGB (red, green, blue) color model (http://www.easyrgb.com/en/). C = Chroma; h° = Hue angle. Values with the same letter ^{a} and ^{b} in each row are not significantly different (p>0.05)

abundance of pigments such as betalains and phenolic compounds (Zitha et al., 2022), which is supported by positive correlations ($p<0.05$) between reducing power and TP ($r = 0.917$), TF ($r = 0.909$), total betacyanins ($r = 0.953$), and betanin ($r = 0.965$) in the flesh. Also, TEAC was positively correlated to betanin ($r = 0.733$). Due to the natural red pigment of the fruit that possibly interferes with the antioxidant assay, the assay used is highly dependent on the reduction or formation of color in the antioxidant reaction (Lu et al., 2021). In addition, without pre-treatment with PGRs, no modification of genes can occur to promote the formation of the betalains and phenolic compounds in the control samples. Therefore, this leads to low antioxidant activity detected in control samples due to the lower amount of bioactive compounds present.

Even though significantly higher ($p<0.05$) antioxidant activities were detected in peels than in fruit flesh, no significant differences were detected in TEAC and reducing power in fruit peels between all treatments, including control samples. This finding is in agreement with the previous finding by Le (2022), who reported that the peels contained a higher amount of radical scavenging compounds than flesh. Attar et al. (2022) revealed that the antioxidant activity presumed for the whole fruit does not always correlate to the concentration of its most abundant betalains. However, it is the consequence of the interaction of all the antioxidant compounds it contains.

Color Characteristics

Color can be considered an important factor for consumer acceptability. The changes in the L*, a*, b*, chroma, and hue angle values were observed to evaluate the effect of different concentrations of PGR on red coloration in red-fleshed dragon fruit. Table 5 shows no significant difference in the *L**, *a**, *b**, chroma, and hue angle values between the control and treated samples with PGR in the fruit flesh and peels. Previously, Öztürk et al. (2013) reported that the MeJA at a concentration of 4,480 mg/L that was applied to 'Fuji' apples promoted the development of red hue, which is opposite to the results obtained in this study.

However, *L**, *a**, *b**, chroma, and hue angle values in peels were relatively higher compared to the flesh. Higher *L** values indicated lower darkness, while higher *a** values showed increased redness. The combination of these values led to higher Chroma and hue angle values in the peels. This result might be due to the abundance of tiny seeds in the flesh fruit that interfered with the color measurement. According to Wu et al. (2019), the seeds may partially contribute to the darkening of fruit flesh, which can be proved by lower *L** values (~29.34 – ~30.22) that indicate higher darkness in the flesh.

In addition, the bract on the fruit peels also contributed to the interference of the peel color measurement, resulting in no significant difference observed in the color changes. As stated by Nguyen et al. (2021), the bract of dragon fruit could

maintain its appearance as 0.1 mM MeJA during postharvest treatment, delaying the degradation of the bract color. This result could result in high L^* values measured in the peel color. Therefore, no correlation was detected between L^*, a^*, b^*, chroma, and hue angle values with betacyanins or other phenolic contents.

Principal Component Analysis (PCA)

The score plot generated from PCA showed a relationship between PGR treatment at different concentrations and the results obtained from the analysis of the dragon fruit flesh and peels, respectively (Figure 3). In the flesh (Figures 3a–3c), the distribution of data analysis is defined by the 75.2%

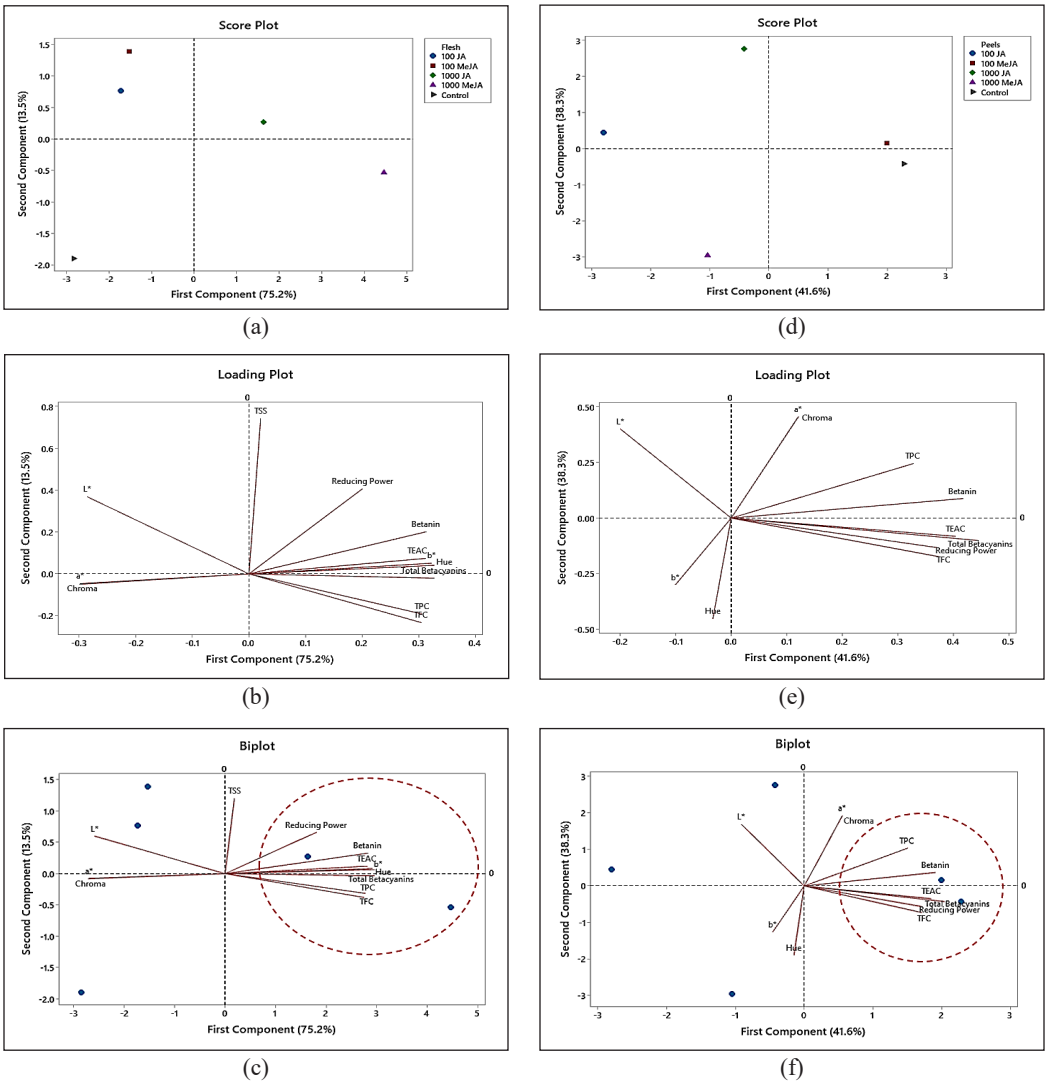

Figure 3. Score plot, loading plot, and bi-plot of principal component analysis based on the effect of two different concentrations of MeJA and JA on the flesh (a–c) and peels (d–f) of red-fleshed dragon fruit
Note. JA = Jasmonic acid; MeJA = Methyl jasmonate

PC1 and 13.5% PC2, where 1,000 ppm PGR, particularly MeJA, was positively correlated to betanin and total betacyanins compound, antioxidant activity, TF, and TP. PGR treatment using 1,000 MeJA efficiently increased the dragon fruit flesh's bioactive compounds and antioxidant activity.

On the other hand, Figures 3d–3f present the loading plot graph, PC1 and PC2 accounted for 41.6 and 38.3%, respectively. Overall, the biplot graph revealed that 100 MeJA and control samples were positively correlated with the betanin and total betacyanins compounds, antioxidant activity, TP, and TF. This finding contradicted the one obtained from the flesh, whereas peels with a lower concentration of MeJA (100 ppm) and untreated dragon fruit showed higher bioactive and antioxidant activity than 1,000 ppm.

CONCLUSION

Bioactive compounds in red-fleshed dragon fruit may vary depending on several factors, such as PGR types, concentrations used, and the interaction between types of PGR and their concentrations. In this study, the application of PGR influenced the accumulation of physicochemical and bioactive substances and antioxidant activities in red-fleshed dragon fruit, particularly the flesh part. PGR treatment using MeJA was more effective than JA. Treatment using 1,000 ppm MeJA effectively increased the total betacyanins, betanin, TSS, TP, TF, and antioxidant activity in the fruit flesh between the concentrations used. However, PGR treatments had little effect on fruit peel. The present correlation in the peels only exhibited between betacyanins, betanin, and TF. In summary, a comprehensive understanding of how JA and MeJA influence red-fleshed dragon fruit can provide valuable insights into plant biology, cultivation practices, and crop management strategies, leading to improved fruit quality, yield, and sustainability in agriculture.

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