Agricultural Sci. J. 50 : 3 (Suppl.) : 23-27 (2019) ว. วิทย. กษ. 50 : 3 (พิเศษ) : 23-27 (2562)

การใช้กรดจิบเบอเรลลิคและเมทิลจัสโมเนตชะลอการสลายตัวของคลอโรฟิลล์ในกลีบประดับของผลแก้วมังกร (*Hylocereus undatus*) ในสภาพการเก็บรักษาที่อุณหภูมิต่ำ

Gibberellic Acid and Methyl Jasmonate Treatments Delay Chlorophyll Degradation in the Bract of Dragon Fruit (*Hylocereus undatus*) in Cold Storage Condition

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Abstract

Dragon fruit is a high-value fruit, showing bright red peel with an appealing green appearance of the bract. Thus, the yellowing of bract is one of major postharvest problems during storage of fresh dragon fruit. This study investigated the effect of gibberellic acid (GA_3) and methyl jasmonate (MeJA) on the changes of bract colour and edible quality of dragon fruit stored in cold condition. Dragon fruit were dipped in 50 $mg.L^{-1}$ GA_3 or 0.1 mM MeJA for 5 min, and then stored at 10°C, 85-90% RH. GA_3 and MeJA suppressed the yellowing of bracts by reducing the loss of chlorophylls by 25.15% and 24.28%, respectively after 30 days of storage. In addition, GA_3 treatment increased total vitamin C content in the pulp of dragon fruit. However, neither GA_3 nor MeJA affected total phenolics content, and DPPH scavenging activity in the pulp as well as disease severity of dragon fruit during storage. We concluded that GA_3 and MeJA could delay chlorophyll degradation in bract and enhanced the edible quality of dragon fruit in cold storage condition.

Keywords: Dragon fruit, Gibberellic acid, Methyl Jasmonate, bract yellowing.

บทคัดย่อ

ผลแก้วมังกรเป็นผลไม้ที่มีคุณค่าสูง มีเปลือกสีแดงแซมด้วยกลีบประดับสีเขียว ดังนั้นการเปลี่ยนเป็นสีเหลืองของกลีบประดับจึงเป็นหนึ่งในปัญหาหลังการเก็บเกี่ยวที่สำคัญระหว่างการเก็บรักษาผลแก้วมังกรสด การวิจัยนี้เป็นการศึกษาผลของกรดจิบเบอเรลลิก และเมทิลจัสโมเนตต่อการเปลี่ยนแปลงสีของกลีบประดับและคุณภาพการบริโภคของผลแก้วมังกรที่เก็บรักษาที่อุณหภูมิต่ำ จุ่มผลแก้วมังกรในกรดจิบเบอเรลลิก ความเข้มข้น 50 $mg{\cdot}L^{-1}$ หรือเมทิลจัสโมเนต ความเข้มข้น 0.1 mM นาน 5 นาที ก่อนนำมาเก็บที่อุณหภูม 10 °C ความชื้นสัมพัทธ์ 85 – 90% กรดจิบเบอเรลลิกและเมทิลจัสโมเนตสามารถยับยั้งการเปลี่ยนเป็นสีเหลืองของกลีบประดับโดยการลดการสูญเสียของคลอโรฟิลล์ถึง 25.15 และ 24.28% ตามลำดับ หลังจากเก็บรักษานาน 30 วัน นอกจากนี้ชุดการทดลองที่ใช้กรดจิบเบอเรลลิกยังมีการเพิ่มขึ้นของวิตามินซีในเนื้อ อย่างไรก็ตาม ทั้งกรดจิบเบอเรลลิกและเมทิลจัสโมเนตไม่มีผลต่อระดับความรุนแรงของการเกิดโรคบนผลแก้วมังกรในระหว่างการเก็บรักษา จึงสรุปได้ว่ากรดจิบเบอเรลลิกและเมทิลจัสโมเนตสามารถชะลอการสูญสลายของคลอโรฟิลล์ในกลีบประดับ และยังเพิ่มคุณภาพการบริโภคของแก้วมังกรที่เก็บรักษาที่อุณหภูมิต่ำได้

คำสำคัญ: แก้วมังกร กรดจิบเบอเรลลิค เมทิลจัสโมเนต

Introduction

Dragon fruit (*Hylocereus undatus*) is one of famed attractive fruits because of the appealing “dragon-like” appearance of bracts. However, the yellowing of bracts occurs quickly after harvest due to the degradation of green pigment chlorophylls during the senescence. GA_3 is a major form of bioactive gibberellins which can control diverse aspects of plant growth and development (Yamaguchi, 2008). Since 1965, GA_3 was proved to inhibit

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senescence in leaf by delaying protein degradation and chlorophyll degradation (Schippers *et al*., 2007). In postharvest, GA_3 can retain chlorophyll content in sugar snap peas (El-hamahmy *et al*., 2017) and banana (Huang *et al*., 2014). On the other hand, MeJA shows different effects on colour changes of fruits and vegetables during the senescence (Kondo, 2005). Exposure to methyl jasmonate vapour 10^{-4} M apparently delayed colour changes of mango fruits cv. Tommy Atkins (Gonzalez-Aguilar *et al*., 2000) while 10^{-5} M methyl jasmonate was reported to promote yellow and red colour development in 'Kent' mango (González-Aguilar *et al*., 2001). However, the effects of both GA_3 and MeJA on the chlorophyll degradation of dragon fruit bracts have not been concerned. Our study aimed to identify the influence of GA_3 and MeJA on the chlorophyll degradation in order to find an approach contributes to maintaining the colour of the bracts of dragon fruit in the postharvest stage.

Materials and methods

1. Fruit materials and experimental design

Dragon fruit (*Hylocereus undatus*) at 80% of maturity stage (the fruit pericarp were bright red over 80% of the surface) were harvested for the experiment. Fruit was sorted by uniform size, colour and freedom of defects before washing by tap water and sanitizing by dipping in 150 $mg.L^{-1}$ sodium hypochlorite for 5 min. After that, dragon fruit was dipped in 50 $mg.L^{-1}$ GA_3 or 0.1 mM MeJA solutions for 5 min. This treatment condition was selected based on different MeJA concentrations (0.01, 0.05, 0.1 mM), different GA_3 concentrations (10, 50, 100 $mg.L^{-1}$), and different treatment duration (5, 25 min) in our preliminary experiment (data not shown). Non-treated fruit was considered as control. Dragon fruit in all treatments then were stored at 10°C, 85-90% relative humidity. Bract colour, chlorophyll content in bracts, disease severity of dragon fruit were evaluated every 5 days; Total soluble solids (TSS), phenolic, vitamin C content, and DPPH inhibition of pulp of dragon fruit were analysed every 10 days.

2. Colour of bracts and chlorophyll content

Colour of bracts: Colour (represented by hue angle h°) of 3 randomly bracts at the upper half of each fruit were measured at the largest surface area by colourimeter (Minolta, model CR–400, Japan).

Chlorophyll content of bracts: Chlorophyll content of bracts was determined using N, N-dimethylformamide (Moran, 1982). Total chlorophyll content was expressed as mg per 100 g of dry weight.

3. Biochemical analysis

Total soluble solids (TSS): An digital refractometer (PAL 1, Atago Co Ltd.) was used to determine the TSS of fruit juice and expressed as percentages.

Titratable acidity (TA): Total acid content of fruit juice was determined by the titration method with 0.1N NaOH solution in the presence of 1% phenolphthalein. TA were expressed as percentages (w/w) of citric acid.

Total phenolic content: Total phenolic content of fruit pulp was determined by Folin-Ciocalteu reagent (Singleton *et al*., 1999). Total phenolic content was expressed as mg gallic acid equivalents (GAE) per 100 g of fresh weight.

Vitamin C content: Total vitamin C was determined by 2,4 dinitrophenyl hydrazine (DNP) method (Roe *et al*., 1948) with some adaptation. Total vitamin C content was expressed as mg per 100 g of fresh weight.

2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity: DPPH assay was performed according to (Li *et al*., 2017). DPPH scavenging activity was expressed by percentage of inhibited DPPH.

Statistical analysis: Analysis of variance (ANOVA) was performed and Duncan's multiple range test was used to compare the measured mean values at each day of sampling.

3. Disease severity

Disease severity of fruit was observed visually and scored according to the rating scale (0: 0% of fruit body surface rotten; 1: 0 < rotten area ≤ 5% of body surface; 2: 5% < rotten area < 10% of body surface; 3: 10% < rotten area < 25% of body surface; 4: 25% < rotten area < 50% of body surface; 5: > 50% rotten area).

Results and discussion

Colour of bracts and the chlorophyll content

As shown in Figure 1, the colour of bract of dragon fruit decreased gradually during storage. However, both 50 mg.L^{-1} GA_3 and 0.1 mM MeJA significantly maintained the green bracts of dragon fruit after 30 days of storage. In accordance with the colour of bracts, chlorophyll content was retained by 25.15% and 24.28% respectively in GA_3 and MeJA treated dragon fruit compared to non-treated fruit after 30 days of storage. The action of GA_3 on chlorophyll preservation was pointed out in many previous studies. GA_3 was proved to prevent the accumulation of senescence-associated genes (SAGs) transcripts and reactive oxygen species (ROS), which results in delaying the chlorophyll breakdown (Rosenvasser *et al*., 2006). Moreover, The activities of enzymes chlorophyllase (Chlase), Mg-dechelatase (MgD) and chlorophyll-degrading peroxidase (Chl-POX) involved in chlorophyll degradation were down-regulated by GA_3 (Li *et al*., 2010). On the other hand, thought the role of MeJA in plant senescence and ripening has not been clearly understood, our results show that MeJA reduced the chlorophyll degradation and maintain the green colour of bract of dragon fruit. MeJA was also found to inhibit the loss of chlorophyll content in bell pepper, along with enhancing antioxidant enzyme activities involved in detoxifying ROS (Wang *et al*., 2019). However, the in-depth study should be investigated to understand the mechanism that GA_3 and MeJA influence the chlorophyll degradation in the bract of dragon fruit.

Changes in pulp quality of fruit

Total soluble solids of dragon fruit decreased gradually during storage, reflecting normal postharvest senescence of fruit (Figure 2A). Neither GA_3 nor MeJA dipping before storage can reduce the loss of TSS. (Mustafa *et al*., 2018) also reported that MeJA could not maintain TSS of dragon fruit.

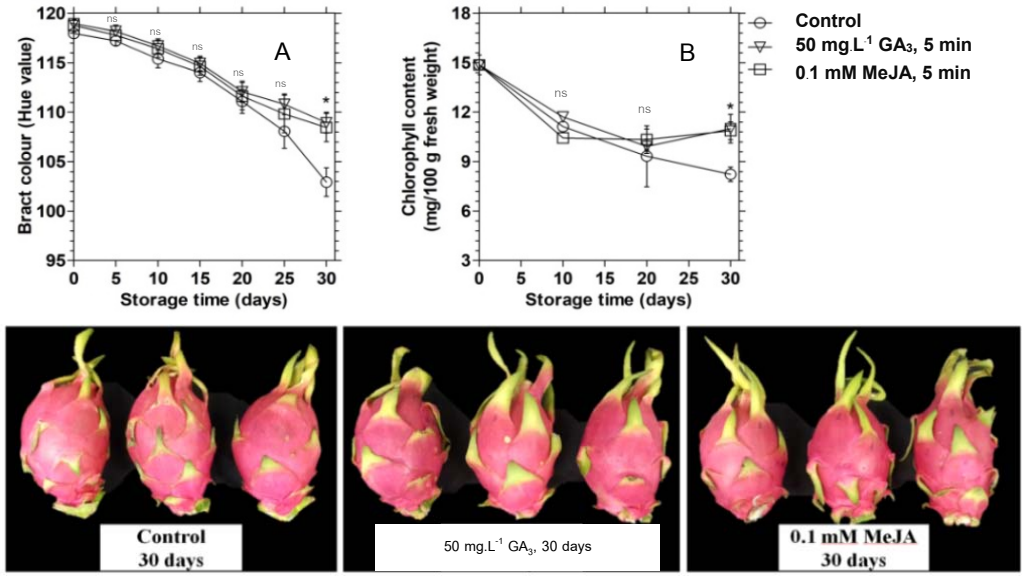

Figure 1. Changes in bract colour - Hue values (A), chlorophyll content in the bracts (B), and visual appearance (C) of dragon fruit pre-treated with 50 mg.L^{-1} GA_3 or 0.1 mM MeJA for 5 min during storage at 10°C. Vertical bars represent standard error (SE) of the mean value.
The symbol (*) = significant difference at $p \leq 0.05$; (ns) = non-significant difference at $p \leq 0.05$.

The significantly higher levels of vitamin C was retained in dragon fruit treated with GA_3 after 20 days of storage (Figure 2C). This is in agreement with the finding of Panigrahi *et al*. (2017) who found that GA_3 delayed the decline in ascorbic acid content in green chilli. MeJA was also reported increasing ascorbic acid content in cherry tomato (Liu *et al*., 2018) and carambola fruit (Mustafa *et al*., 2016). However, there was no significance on phenolic content and DPPH inhibition capacity of dragon fruit between non-treated fruits and GA_3 or MeJA treated fruit.

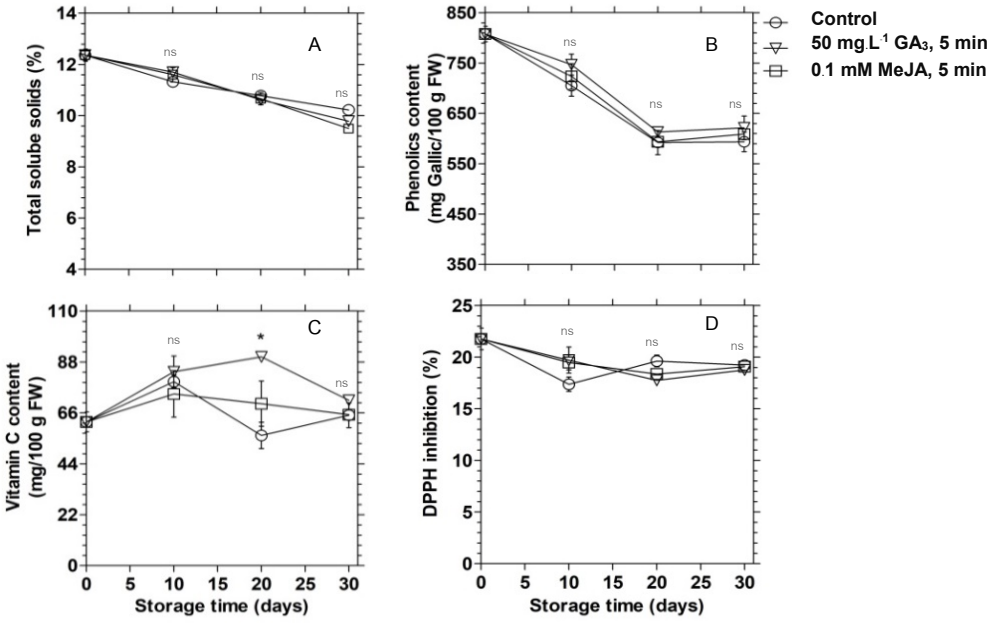

Figure 2. Changes in total soluble solids (A), phenolic content (B), vitamin C content (C) and DPPH inhibition (D) of dragon fruit pre-treated with 50 mg.L^{-1} GA_3 or 0.1 mM MeJA for 5 min during storage at 10°C. Vertical bars represent standard error (SE) of the mean value.
The symbol (*) = significant difference at $p \leq 0.05$; (ns) = non-significant difference at $p \leq 0.05$.

Disease incidence and severity of dragon fruit

There were no disease symptoms found on dragon fruit by 20 days of storage. After 30 days of storage, the lowest mean score of disease severity was found in GA_3 treated dragon fruit (Figure 3). However, there was no significant difference in disease severity of dragon fruit between treatments.

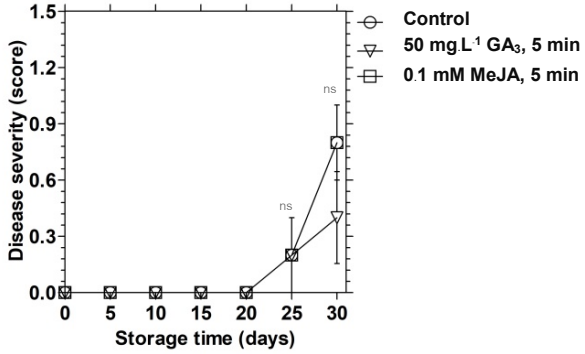

Figure 3. Changes in disease severity of dragon fruit treated with 50 mg.L^{-1} GA_3 or 0.1 mM MeJA for 5 min during storage at 10°C. Vertical bars represent standard error (SE) of the mean value.
The symbol (*) = significant difference at $p \leq 0.05$; (ns) = non-significant difference at $p \leq 0.05$.

Conclusions

In conclusion, both GA_3 and MeJA are positive approaches to maintaining the chlorophylls in bracts of dragon fruit. However, to provide a higher effect on all attributes of dragon fruit, the other technique should be investigated to combine with GA_3 or MeJA.

Acknowledgments

This study was funded by Petchra Pra Jom Klao Doctoral Scholarship (No. of agreement 25/2560), King Mongkut's University of Technology Thonburi. The authors thank to The United Graduate School of Agricultural Science (UGSAS), Gifu University, Japan for supporting some equipment in this study.

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