RIPENING EFFECT ON COLOR INDICES, POLYPHENOL CONTENTS, AND ANTIOXIDANT ACTIVITIES OF GUAVA (*Psidium guajava* L.) FRUITS

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ABSTRACT

Guava (*Psidium guajava* L.), a tropical plant, is used as food and medicine to cure certain illnesses. This study aimed to examine changes in physiological indices, phenolic contents, and antioxidant activities of two guava cultivars, cv. Dong Du and cv. Dai Loan, during ripening. Guava fruits were harvested at four different stages, namely mature green, color turning, ripe, and overripe. Phenolic content and antioxidant activity were determined using the Folin-Ciocalteu and DPPH methods, respectively. The results showed that during maturation, the diameter and weight of Dong Du cultivar significantly increased, while these variables of the Dai Loan cultivar did not significantly change. Regarding the color index, the L, a, and b values increased, whereas the hue value decreased in both cultivars during fruit ripening. Ascorbic acid content increased during ripening and no difference was found between two cultivars. The phenolic content and antioxidant activity declined during maturation and these values were higher in the Dong Du cultivar compared to the Dai Loan cultivar. The phenolic content and the antioxidant acitivity were strongly correlated (P <0.001), indicating that the phenolic compounds were the major antioxidants in guava fruits. The ripe stage would be a suitable time to harvest fruits for consumption of guava products.

Keywords: Phenolic compounds, color index, *Psidium guajava* L., ripening.

Ảnh hưởng của thời kỳ chín đến đến chỉ số màu sắc, hàm lượng polyphenol và hoạt tính kháng ôxy hóa của quả ổi (*Psidium guajava* L.)

TÓM TẮT

Ổi (*Psidium guajava* L.) là một loại cây nhiệt đới, được sử dụng làm thực phẩm và thuốc để chữa một số bệnh. Nghiên cứu này nhằm đánh giá sự biến đổi về chỉ số sinh lý, hàm lượng phenolic và khả năng kháng oxy hóa trong quá trình chín của hai giống ổi, Đồng Du và Đại Loan. Quả ổi được thu hoạch ở bốn giai đoạn khác nhau, giá sinh lý, chuyển màu, chín và chín quá. Hàm lượng phenolic và hoạt tính kháng oxy hóa được xác định bằng phương pháp Folin-Ciocalteu và DPPH. Kết quả cho thấy trong quá trình chín, đường kính và trọng lượng của giống ổi Đông Du tăng đáng kể, trong khi các biến số này của giống Đại Loan không thay đổi đáng kể. Về chỉ số màu sắc, các giá trị L, a và b tăng lên, trong khi giá trị hue giảm ở cả hai giống trong quá trình chín của quả. Hàm lượng acid ascorbic tăng trong quá trình chín và không có sự khác biệt giữa hai giống. Hàm lượng phenolic và hoạt tính kháng oxy hóa giảm trong quá trình chín và hai chỉ số này cao hơn ở giống Đông Du so với giống Đại Loan. Hàm lượng phenolic và khả năng chống oxy hóa có mối tương quan chặt (P <0,001), cho thấy các hợp chất phenolic là chất kháng oxy hóa chính trong ổi. Giai đoạn chín là thời điểm thích hợp để thu hoạch trái cây để tiêu thụ để. Thời gian thu hoạch phù hợp là rất quan trọng để sản xuất các sản phẩm ổi chất lượng cao như nước ép ổi và mứt.

Từ khóa: Hợp chất phenolic, chỉ số màu, *Psidium guajava* L., chín.

1. INTRODUCTION

Oxidative stress imposed by the overproduction of reactive oxygen species (ROS), or free radicals, in the body can result in health problems such as cancers, degenerative diseases, and aging (Ahmed, 2005). These ROS and free radicals are capable of damaging the biological structures of lipids, proteins, and nucleic acids (Li *et al.*, 2007; Fu *et al.*, 2011). Therefore, the prevention of excessive oxidation processes is crucial for maintaining human health. Although

humans have evolved with an antioxidant defense system against free radicals and ROS, antioxidants produced in the body are inadequate due to their exposure to free radicals from external sources in the modern world (Di Mascio *et al.*, 1991; Duan *et al.*, 2007). Thus, it is necessary to supplement antioxidants from external sources in order to protect the body against the consequences of oxidative stress (Arabshahi-Delouee & Urooj, 2007). Polyphenols, carotenoids, and fatty acids are natural antioxidants, of which polyphenols have been attracting interest due to their applications in cosmetics, nutraceuticals, and pharmaceuticals (Handique & Baruah, 2002; Rao & Rao, 2007).

Polyphenols are organic compounds characterized by the presence of phenol units, which are substituted with hydroxyls, that are widely distributed in plants and important to human health (Petti & Scully, 2009). Research has shown that polyphenols reduce the risk of particular cancers such as liver, cervical, and colon cancer (Kim *et al.*, 2006; Di Domenico *et al.*, 2012; Stagos *et al.*, 2012). In fact, polyphenols have shown their potential to prevent the carcinogenesis process by acting on intracellular signaling molecules involved in the initiation of cancer (Link *et al.*, 2010). Besides, polyphenols possess the capability to inhibit the development of cardiovascular disease (Vauzour *et al.*, 2010). Leifert & Abeywardena (2008) stated that grape polyphenols can play an important role in preventing cardiovascular disease. Another study on people with high cardiovascular risk showed that a high intake of polyphenol-rich foods may lead to reducing one's cardiovascular risk (Medina-Remón *et al.*, 2011). Also, polyphenolic compounds exhibit effects against neurodegenerative disorders such as Parkinson's and Alzheimer's diseases (Ebrahimi & Schluesener, 2012). These compounds protect humans from neurodegenerative processes by acting on different molecular targets including ROS regulation (Choi *et al.*, 2012). Lau *et al.* (2005) found that polyphenols from blueberries can reverse neuronal signal transduction declines as well as preserve cognitive performance with aging.

Fruits are the primary dietary source of polyphenols for humans; therefore, their phenolic compounds and antioxidant capacities have been studied in recent years (Kherwar and Usha, 2016; Hernández *et al.*, 2017). Research has shown that fruits are high in phenolic contents and antioxidant activities, which can vary depending on the fruit (Rufino *et al.*, 2010; Fu *et al.*, 2011). Besides, there are significant differences in the contents of phenolic compounds and antioxidant capacities of fruits at different stages of their maturation. For example, the total phenolic content and the antioxidant activity of tomato increased during ripening (Ilahy *et al.*, 2011), while these values reduced during the maturation of other fruits such as medlar *(Mespilus germanica L.)* and *Rubus coreanus* (Park *et al.*, 2008; Gruz *et al.*, 2011). Guava fruits (*Psidium guajava* L.), which are commonly available in tropical and sub-tropical regions, exhibit high values of total phenolic compounds and antioxidant capacity (Contreras-Calderón *et al.*, 2011). Guava can be consumed at various stages of maturity; therefore, it is necessary to evaluate nutritional values among ripening stages. This study aimed to investigate changes in total phenolic content and antioxidant activities of two guava cultivars grown in Vietnam at different stages of their maturation.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

DPPH (1,1-diphenyl-2-picryl-hydrazyl) and Trolox were of analytical-grade and purchased from Sigma (USA). Gallic acid and Folin-Ciocalteu reagent were purchased from Merck (Germany).

2.2. Sample collection

Guava fruits of the Dong Du cultivar and Dai Loan cultivar were collected from guava trees in the Trau Quy commune, Gia Lam district, Hanoi, Vietnam. The fruits were divided into four different ripening stages (RS) based on their skin color, namely mature green (100% green, RS1), color turning (80% green and 20% yellow, RS2), ripe (50% green and 50%

yellow, RS3), and overripe (20% green and 80% yellow, RS4) (Jain *et al.*, 2003). At each maturity stage, five and ten fruits of the Dai Loan and Dong Du cultivars, respectively, were selected. The skin color, weight, diameter, and volume of the fruits were measured. After the physiological variables were measured, the fruits were cut into 8 pieces and symmetrical pieces of fruits were collected in 4 zip plastic bags and stored at -56°C for further analyses.

2.3. Skin color, fruit diameter, and spacific gravity measurements

Skin color was measured using a colorimeter (CR-400, Konica Minolta, Japan, illuminant C) on three different sites of each fruit. The color parameters were expressed in L, a, and b values. The L value represented the lightness and this value increased as the color changed from black to white. The a value indicated the redness and this value increased as the color changed from green to red. The b value showed the yellowness and it increased as the color changed from blue to yellow. The hue value was calculated using the following equation described by Jha (2010).

$$\text{hue} = 180 + \frac{\text{Actan}\left(\frac{b}{a}\right) * 360}{6.2832}$$

The diameters of the fruits were determined using a caliper. At each ripening stage, 10 and 5 fruits were measured for the Dong Du and Dai Loan cultivars, respectively, and each fruit was measured at three positions.

Specific gravity was measured as the fruit density divided by the water density. The fruit density was measured as the ratio of the fruit weight to the fruit volume (g/mL). The fruit weight was measured by a technical balance and the fruit volume was measured by the volume of water that the fruit occupied.

2.4. Total soluble solids (TSS) determination

Total soluble solids (TSS) were determined for each ripening stage using a digital refractometer (PR-101, Atago, Japan) and expressed in °Brix. TSS at each maturity stage was measured in triplicate.

2.5. Determination of ascorbic acid content

The methods of Musulin and King (1936) were used to extract ascorbic acid. Five grams of guava fruit were homogenized and extracted with 50 mL of HPO_3 0.2% and CH_3COOH 8% buffer. Ascorbic acid content was then determined by titration with I_2 0.01 N. A standard curve was also made with the five working solutions of 0, 0.5, 1.0, 1.5, and 2.0 mg/mL for the calculation of the ascorbic acid content in the extracted samples.

2.6. Fruit extraction for the determination of phenolic content and antioxidant capacity

Fruit extracts for the determination of the total phenolics content and antioxidant activity were prepared using methods adapted from Alothman *et al.* (2009) with slight modifications. Five grams of guava fruit were ground using a pestle and then mixed with 25 mL of acetone 90%. The crude extract was kept for 3h in the dark at room temperature and regularly shaken using a vortex every 10 minutes. The extract was then centrifuged at 6000 rpm for 20 minutes and the supernatant was collected and stored at -23°C. Fruit extraction at each maturity stage was carried out in triplicate.

2.7. Determination of total phenolic content

Total phenolic content was measured using the Folin-Ciocalteu method described by Fu *et al.* (2011). In brief, 0.5 mL of a diluted sample was transferred into a test tube and 2.5 mL of 1:10 diluted Folin-Ciocalteu reagent was then added and mixed well. After 4 minutes, 2 mL of 7.5% Na_2CO_3 was added. The reaction was incubated at room temperature in the dark for 2h and the absorbance of the mixture was then measured at 760 nm using a UV-visible spectrophotometer (Lambda 25, Perkin Elmer, USA). The results were expressed in mg gallic acid equivalents/100 g fresh weight (mg GAE/100 g FW; FW: fresh weight) using a gallic

acid standard curve. A stock solution of 1 mg/mL gallic acid was prepared and the calibration curve was then established based on the working-standard solutions of 0.02, 0.04, 0.06, 0.08, and 0.1 mg/mL.

2.8. Antioxidant capacity measurement

Antioxidant capacity was determined using the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay described by Thaipong *et al.* (2006) with several modifications. The stock solution of DPPH was made by dissolving 24 mg of DPPH into 100 mL of methanol and the solution was kept at -23°C until needed. The working solution of DPPH was prepared by diluting 10 mL of the stock solution into 45 mL of methanol. The reaction was carried out by mixing 150 µL of diluted fruit extract with 2,850 µL of the DPPH solution for 30 minutes. A control was also prepared by using 150 µL of methanol instead of the fruit extract. The absorbance was then measured at 515 nm using a spectrophotometer (Lambda 25, Perkin Elmer, USA). The results were expressed in Trolox equivalents (µM TE/g FW) using a Trolox standard curve. The standard curve was established based on the Trolox standard solutions of 50, 100, 250, 500, 750, and 1,000 µM.

2.9. Statistical analysis

Data were analyzed using R version 3.4.3. Tukey's test was used for the comparison of the means. Correlations among variables were assessed using Pearson's correlation coefficient (r).

Table 1. Effect of cultivar and ripening stage on fruit diameter, fruit weight, specific density, total soluble solids (TSS), and ascorbic acid content

	df	*F* ratio				
		Diameter	Weight	Specific density	TSS	Ascorbic acid content
Cultivar (C)	1	756.92***	669.92***	3.54 ns	3173.44***	0.273 ns
Ripening stage (RS)	3	23.56***	4.61**	5.85**	502.63***	476.77***
C*RS	3	18.43***	4.40**	0.35	58.78***	11.27***

*Note: F ratios are from two-way ANOVA analysis. **and *** indicate significance at P <0.01 and P <0.001, respectively; ns: not significant.*

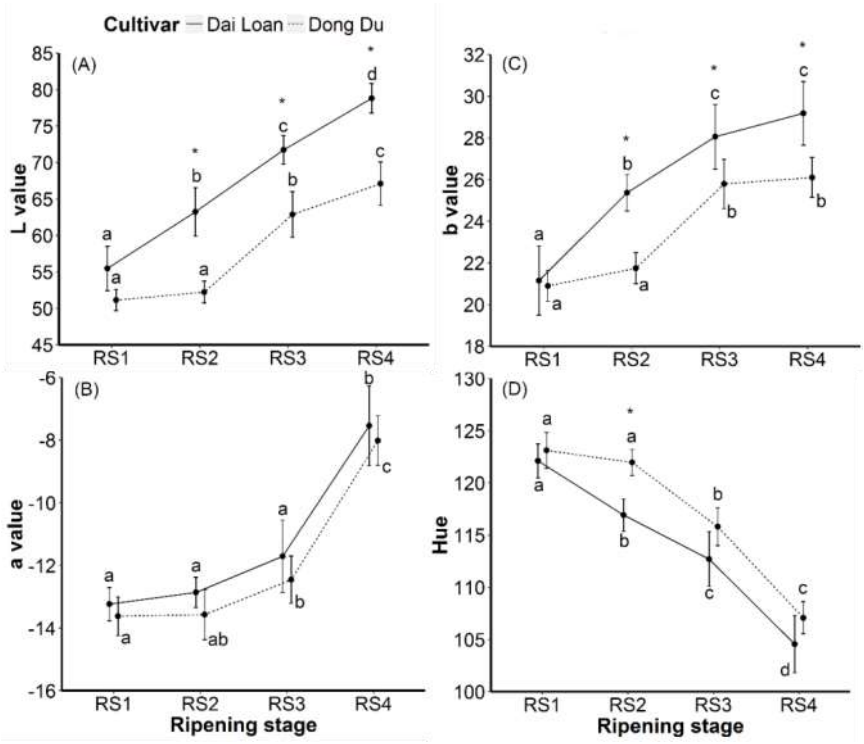
Table 2. Changes in diameter, weight, specific gravity, total soluble solids (TSS), and ascorbic acid content in two guava cultivars at four ripening stages

Variable	Cultivar	Ripening stage				
		RS1	RS2	RS3	RS4	Mean
Diameter (cm)	Dong Du	4.23*^{a} ± 0.10	4.68*^{b} ± 0.10	4.68*^{b} ± 0.06	5.64*^{c} ± 0.20	4.78
	Dai Loan	6.89^{a} ± 0.45	7.17^{a} ± 0.14	6.91^{a} ± 0.48	6.79^{a} ± 0.66	6.94
Weight (g)	Dong Du	48.80*^{a} ± 0.52	59.93*^{ab} ± 3.62	59.95*^{ab} ± 3.24	84.72*^{b} ± 8.85	62.8
	Dai Loan	177.35^{a} ± 31.52	198.46^{a} ± 12.03	186.10^{a} ± 36.97	177.39^{a} ± 32.11	177.64
Specific gravity (g/mL)	Dong Du	0.99^{a} ± 0.14	0.90^{ab} ± 0.10	0.86^{b} ± 0.09	0.85^{b} ± 0.08	0.90
	Dai Loan	1.03^{a} ± 0.11	0.91^{a} ± 0.06	0.95^{a} ± 0.04	0.91^{a} ± 0.02	0.95
TSS (°Bx)	Dong Du	7.60*^{a} ± 0.10	8.33*^{b} ± 0.06	8.90*^{c} ± 0.00	9.33*^{d} ± 0.06	8.54
	Dai Loan	6.67^{a} ± 0.06	7.07^{b} ± 0.06	7.23^{b} ± 0.06	7.57^{b} ± 0.06	7.13
Ascorbic acid content (mg/100 g FW)	Dong Du	72.75^{a} ± 0.15	89.86^{b} ± 1.22	112.93*^{c} ± 1.80	147.37^{d} ± 3.74	106.50
	Dai Loan	82.23^{a} ± 2.46	88.43^{ab} ± 3.17	100.77^{c} ± 2.83	154.05^{d} ± 7.78	105.73

Table 3. Effect of cultivar and ripening stage on the color indices (L, a, b, and hue) of the two guava cultivars

	df	*F* ratio			
		L	a	b	hue
Cultivar (C)	1	182.72***	9.29**	60.47***	40.69***
Ripening stage (RS)	3	174.81***	152.34***	103.32***	235.68***
C*RS	3	5.96***	0.16 ns	5.89**	2.83*

*Note: F ratios are from the two-way ANOVA analysis. *, **, and *** indicate significance at P <0.05, P <0.01, and P <0.001, respectively; ns: not significant.*



*Note: Data represent means ± standard deviations (n = 10 for the Dong Du cultivar, n = 5 for the Dai Loan cultivar). *: significant (P <0.05) difference between the two cultivars at each ripening stage. Different letters show significant (P <0.05) differences between the ripening stages of each cultivar.*

Figure 1. Changes in the color indices of the two guava cultivars during ripening

3. RESULTS

3.1. Changes of physiological indices during fruit ripening

Significant (P <0.01) interactions between the cultivar (C) and ripening stage (RS) for fruit diameter and fruit weight were observed. The results in Table 1 indicate that the fruit diameter and the fruit weight of the Dong Du cultivar significantly increased over the course of ripening from 4.23 to 5.64 cm and from 48.80 to 84.72 g/fruit, respectively (Table 2), while these indices of the Dai Loan cultivar showed no changes.

The Dai Loan cultivar showed a greater fruit diameter and fruit weight than the Dong Du cultivar at all the ripening stages. On average, the Dai Loan cultivar was 1.5 times larger in fruit diameter than the Dong Du cultivar, and the fruit weight of the Dai Loan cultivar was about 3 times greater (Table 1).

There was no significant C x RS interaction for specific density and no significant difference in this variable was observed between the two cultivars (Table 1). However, during ripening, specific gravity significantly (P <0.01) decreased for both cultivars (Tables 1 and 2).

A significant (P <0.001) C x RS interaction for TSS was observed (Table 1). TSS increased in both cultivars during ripening, but the increase in the Dai Loan cultivar was less significant. The TSS of the Dong Du cultivar was significantly (P <0.05) higher than that of the Dai Loan cultivar at all the ripening stages (Table 2). This value of the Dong Du cultivar increased from 7.6 oBx at RS1 to 9.3° Bx at RS4, while it enhanced from 6.7-7.6° Bx in the Dai Loan cultivar.

Data represent means ± standard deviations (diameter, weight, and specific density: n = 10 for the Dong Du cultivar, n=5 for the Dai Loan cultivar; TSS and ascorbic acid content: n = 3). *: significant (P <0.05) differences between the two cultivars at each ripening stage. Different letters show significant (P <0.05) differences between the ripening stages of each cultivar.

A significant (P <0.001) C x RS interaction for ascorbic acid content was also found (Table 1). The results from Table 2 show that the Dong Du cultivar had greater (P <0.05) ascorbic content at RS3 than the Dai Loan cultivar, but no significant differences were observed at the other ripening stages. The ascorbic acid content significantly increased in both cultivars during maturation. Indeed, this value of the Dong Du cultivar doubled at RS4 (147.37 mg/100 g FW) compared to RS1 (72.75 mg/100 g FW), and for the Dai Loan cultivar it increased by 1.9 times to 154.05 mg/100 g FW at RS4 from 88.23 mg/100 g FW at RS1 (Table 2).

Significant (P <0.05) C x RS interactions for the L, b, and hue indices were observed, while this interaction for the a index did not occur (Table 3). The Dai Loan cultivar exhibited higher L, a, and b values than the Dong Du cultivar (Figure 1A, B, C) while the hue value was lower in the Dai Loan cultivar (Figure 1D). The ripening stage significantly affected all the color indices. In fact, during fruit ripening, the L, a, and b values steadily increased (Figure 1A, B, C), while the hue angle gradually decreased in the two cultivars (Figure 1D). However, the patterns of changes were slightly different between the two cultivars as shown in Figure 1; the hue value of the Dong Du cultivar reduced more dramatically than that of the Dai Loan cultivar from RS1 to RS2. The increases of the L, a, and b values indicated that fruit color changes from green to yellow when guava ripens.

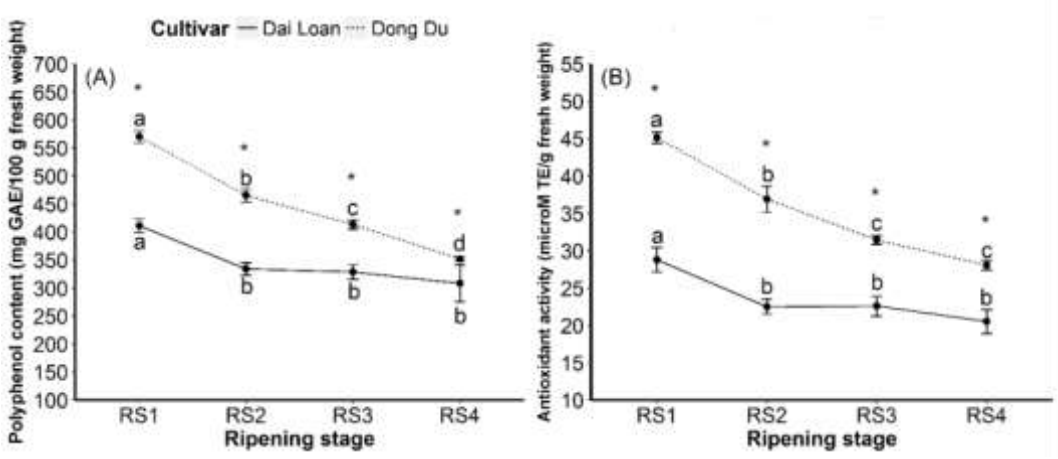
3.2. Changes of phenolic content and antioxidant activity during fruit maturation

A significant (P <0.001) C x RS interaction for phenolic content, as shown in Table 4, explains the differences between the patterns of changes in phenolic contents during the ripening of the two guava cultivars. Indeed, the phenolic content of the Dong Du cultivar steadily decreased from 570.2 mg GAE/100 g FW at RS1 to 351.6 mg GAE/100 g FW at RS4, whereas this value significantly declined from 411.0 mg GAE/100 g FW at RS1 to RS2 then slightly decreased to 308.3 mg GAE/100 g FW at RS4 in the Dai Loan cultivar (Figure 2A).

Table 4. Effect of cultivar and ripening stage on the polyphenol content and antioxidant activity of the two guava cultivars

	df	*F* ratio	
		Polyphenol content	Antioxidant activity
Cultivar (C)	1	282.88***	548.7***
Ripening stage (RS)	3	120.27***	117.0***
C*RS	3	16.94***	17.8***

*Note: F ratios are from the two-way ANOVA analysis. *** significant at P <0.001.*



*Note: Data represent means ± standard deviations (n = 3). *: significant (P <0.05) differences between the two cultivars at each ripening stage. Different letters show significant (P <0.05) differences between ripening stages of each cultivar. GAE: gallic acid equivalent, TE: Trolox equivalent.*

Figure 2. Changes in polyphenol content and antioxidant activity in the two guava cultivars at different ripening stages

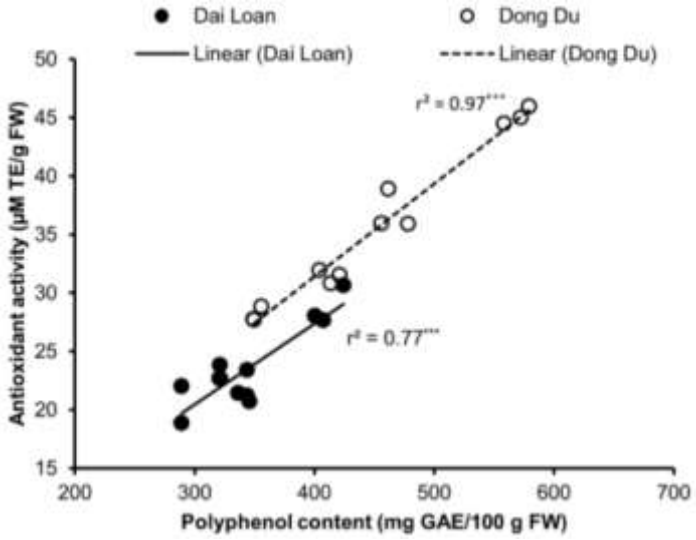


Figure 3. Correlation between polyphenol content and antioxidant activity of the two guava cultivars

*Note: ***: significantly correlated at P <0.001. FW: fresh weight.*

The phenolic concentration of the Dong Du cultivar was higher than that of the Dai Loan cultivar at all the ripening stages. This value of the Dong Du cultivar at RS1 demonstrated a 28% greater phenolic concentration than the Dai Loan cultivar, and the difference was less significant during ripening (Figure 2A).

The Dong Du cultivar had greater antioxidant activities than the Dai Loan cultivar through the four ripening stages. The difference between the two cultivars was more significant at RS1 and then it became less significant as the fruits continued to ripen (Figure 2B). The antioxidant activities declined in both cultivars during fruit ripening and the decrease was more significant in the Dong Du cultivar. At RS4, the antioxidant capacity of the Dong Du cultivar was 28.1 µM TE/g FW, a reduction of approximately

38% compared to that at RS1, while this reduction was just 28% in the Dai Loan cultivar from 28.8 to 20.5 µM TE/g FW (Figure 2B). The results also showed that the polyphenol content was strongly correlated (r^2 = 0.97, P <0.001 and r^2 = 0.77, P <0.001 for the Dong Du and Dai Loan cultivars, respectively) with antioxidant activity (Figure 3).

4. DISCUSSION

Differences in guava fruit diameter and weight have been observed in various varieties. For example, as reported by Brown and Wills (1983), six guava varieties collected in Queensland, Australia significantly differed in weight, which varied from 106.5 to 405.5 g. Variations in size were also observed in varieties originating from different countries including Malaysia, Vietnam, and Taiwan; their diameters ranged from 4.8 to 11.0 cm (Yusof, 1990).

In general, increases in fruit size and weight are a common trend during fruit maturation. Yusof and Mohammed (1987) reported that the weight of a Vietnamese variety increased during maturation. A similar increasing pattern was also observed in other varieties (Brown and Wills, 1983). To the authors' knowledge, the consistency in fruit size and weight through the four stages of maturity as observed in the Dai Loan cultivar in this research has not been reported and this may be an interesting characteristic of this cultivar.

The decrease in specific gravity over the course of ripening in this study displayed a similar trend to other studies (Tandon *et al.*, 1989; Mercado-Silva *et al.*, 1998). Stahl (1933) also found that specific gravity declined during the maturation of avocado fruits. The specific gravity could be used to evaluate the maturity index (Stahl, 1933; Yusof and Mohamed, 1987). Tandon *et al.* (1989) reported that guava fruits with a specific gravity < 1 were more acceptable for harvest, while fruits with a specific gravity > 1.2 should not be harvested since they were still immature. However, using specific gravity as a criterion of maturity is limited because the differences in specific gravity between maturity stages are small and there were large variations between samples. Therefore, this should be used to accompany other indices such as firmness or skin color.

Similar to the results in this study, the variation in TSS among guava varieties has been reported in previous studies (El Bulk *et al.*, 1997; Mercado-Silva *et al.*, 1998). Bashir and Abu-Goukh (2003) reported that the TSS gradually increased in two guava varieties, white and pink guava fruits during maturation_ENREF_37, while the TSS of guava fruit harvested in the spring-summer season increased during the maturation of the fruit (Mercado-Silva *et al.*, 1998). The conversion of starch to sugars could result in the observed increases in TSS during the maturation of fruits (Bashir and Abu-Goukh, 2003). El Bulk *et al.* (1997) indicated that the total sugar content significantly increased during fruit ripening of various guava varieties. Another study also confirmed an increase in total sugar content and a decrease in starch content (Jain *et al.*, 2001).

Ascorbic acid is an important compound since it acts as an antioxidant. This study showed that ascorbic acid content increased in both guava cultivars during ripening. This agrees with the results of 15 guava genotypes reported by Dantas *et al.* (2013). In the study of Dantas *et al.* (2013), the ascorbic acid content at the complete yellow stage varied from 14.38-94.84 mg/100 g FW, which was lower than the values measured in the Dong Du and Dai Loan cultivars, 147.37 and 154.05 mg/100 g FW, respectively. However, in another study, the ascorbic acid content of guava ranged from 174.2-278.5 mg/100 g. These differences could be due to genotypic variation.

Increases in the L, a, and b values, but a decline in the hue value, occurred during ripening. Similarly, Mercado-Silva *et al.* (1998) also found that the color indices of guava fruits grown in Mexico had the same pattern as in this study. The hue value also decreased in other guava cultivars (Cavalini *et al.*, 2006). The alteration in fruit color is due to the changes of

pigments during the maturation of fruits. Increases of total carotenoid content and decreases of total chlorophyll content were observed during guava maturation (Jain *et al.*, 2001). These changes result in the development of color from green to yellow. The color index is a valuable reference to assess the maturity of fruits since this is a nondestructive and time-saving measurement method. Color values were also used to develop nondestructive methods for the evaluation of other fruits, such as dragon fruits and apples (Wanitchang *et al.*, 2010; Iglesias *et al.*, 2012).

The significant differences in phenolic content between the two cultivars, Dong Du and Dai Loan, could be due to genotypic variation. Thaipong *et al.* (2006) reported that total phenolics varied from 170-345 mg GAE/100 g FW among guava genotypes at maturity and this agrees with our results where the phenolic contents of the Dong Du and Dai Loan cultivars were 351.6 and 308.3 mg GAE/100 g FW at RS4, respectively. Phenolic content and fruit size may be negatively correlated. In fact, the phenolic content of guava fruits was higher in peels compared to pulp (Marquina *et al.*, 2008). As a result, larger guava fruits, such as the Dai Loan cultivar, tend to have a lower content of phenolic compounds because the proportion of peels/whole fruits seems to be smaller in larger guava fruits. However, this hypothesis needs to be further evaluated.

The observed decrease in phenolic content during fruit ripening could be attributed to the conversion of phenolic compounds. According to Goldstein and Swain (1963), this decline was associated with the increased polymerization of leucoanthocyanidins during fruit maturation. The polymerization not only affects extractability, but also influences chemical reactivity due to the fact that the polymerization involves the formation of linkages between reactive positions of monomers. Besides, the downward trend of phenolic content during fruit maturation could be influenced by the dilution of polyphenols as the fruits enlarge (Lakshminarayana *et al.*, 1969). This could explain why the decrease of phenolic content in the Dong Du cultivar was more significant than that of the Dai Loan cultivar. The decline of phenolic content during guava maturation was in agreement with other studies (El Bulk *et al.*, 1997; Bashir and Abu-Goukh, 2003; Gull *et al.*, 2012). Similar findings were also observed in other types of fruits, such as medlar (*Mespilus germanica* L.) and myrtle (*Myrtus communis* L.) during their maturation (Fadda and Mulas, 2010; Gruz *et al.*, 2011).

Similar to the phenolic content, antioxidant activity declined with the progress of fruit ripening. At RS4, the antioxidant activities of the Dong Du and Dai Loan cultivars were 28.1 and 20.5 µM TE/g FW, respectively. These results were in agreement with the report by Thaipong *et al.* (2006) in which antioxidant activity ranged from 16.2-32.0 µM TE/g FW among guava genotypes.

The strong and positive correlation between phenolic content and antioxidant activity ($P < 0.001$) (Figure 3) indicated that total phenolic content was a major component accounting for antioxidant capacity. In fact, phenolic compounds are known as the most important antioxidants of fruits (Fu *et al.*, 2011; Sulaiman *et al.*, 2011). Significant correlations between phenolic content and antioxidant capacity were also found in myrtle and medlar fruits during their maturation (Fadda and Mulas, 2010; Gruz *et al.*, 2011). Similarly, this strong relationship was also observed in other fruit sources (Javanmardi *et al.*, 2003; Orak, 2007). The loss of antioxidants during fruit maturation leads to a lower bioactive capacity. Therefore, harvesting fruits at the appropriate maturity stage is important to obtain the highest bioactive capacity.

Although high phenolic content as well as high antioxidant capacity were observed at the mature green and color turning stages, it is not recommended that fruits be harvested at this stages due to their astringency. At the overripe stage, antioxidants were significantly lost; thus, the value of these fruits was reduced. Besides, overripe fruits are too soft to eat and they would

be difficult to preserve. Ripe fruits contain a relatively high ascorbic acid content appear to be the best for consumption or for use in the production of guava products. Further investigation on the physio-chemical properties (i.e. texture and firmness) and flavors of fruits at this ripening stage should be carried out to find out whether they are suitable for industrial production. Guava fruits harvested at a suitable stage can be used as good materials for producing high quality guava products such as guava juice and jam.

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