Original Research Article

Varietal Differences Among the Phenolic Contents and Antioxidant Activities of White and Red Fleshed Guava During Maturation and Ripening Stages

ABSTRACT

Guava (*Psidium guajava* L.) is an important commercial fruit in southern China. In this study, the antioxidant activity and total contents of phenolics and flavonoids of two guava cultivars that with different pulp color were examined. The contents of total phenolics and flavonoids increased during maturation in guava fruit. And red-flesh guava showed higher contents of total phenolics and flavonoids than white-flesh guava. Both DPPH and FRAP assays indicated that antioxidant activity increased during fruit maturation. Strong and positive correlations between total phenolics, total flavonoids and antioxidant activity were observed in our study. Our results suggest that the antioxidant activity and contents of total phenolics and flavonoids in *P. guajava* vary significantly among different cultivar and pulp color.

Keywords: Pisdium guajava; guava; phenolic; antioxidant capacity

1. INTRODUCTION

Among tropical and subtropical fruit, guava (*Pisdium guajava* L.) is considered a good source of vitamins and is generally known as 'Apple of Tropics' [1]. It is cultivated in all tropical and subtropical countries nowadays. Guava belongs to the family *Myrtaceae*, and more than 150 species distributed and cultivated around the world [2]. In general, the peel color of guava fruit usually changes from green to yellow. While, the flesh of guava fruit mostly varies depending on the cultivar or species, and most cultivars are white-fleshed. Two guava cultivars with different flesh color, namely white-fleshed and red-fleshed were cultivated widely in tropical and subtropical regions in China. Guava fruits become soft, edible, and produce an intense aroma in parallel with ethylene production during maturation and ripening. The consumption of guava fruit has been increasing in recent years mainly due to its unique flavor and richness in vitamins.

Phenolic compounds are a kind of ubiquitous secondary metabolites and comprised by flavonoids, which account for about two thirds of the total phenols, and phenolic acids [3]. Phenolics are also known to act antioxidants because of their ability to donate hydrogen or electrons to the free radical and convert it into an innocuous molecule [4]. It has been

reported in many studies that phenolic compounds in various fruit could avoid the oxidative damage by scavenging the free radicals from cell metabolism [5, 6]. The content of phenolics in fruit is dependent on many factors such as degree of maturation, cultivar, soil composition, geographic location and storage conditions [7]. The flavonoids distributed widely in the plant kingdom and account for two-thirds of the total phenols [8]. Flavonoids are usually described as broad collection of compounds with a polyphenol diphenylpropane (C6-C3-C6) skeletons and also with a huge diversity of structures. There are at least four major classes, namely 4-oxoflavonoids (flavones, flavonols), anthocyanis, isoflavones, and flavan-3-ol derivatives (catechin and tannins) [9]. There are at least six methods that have been used to evaluate and compare the antioxidant activity arise from the phenolics in fruit. The most commonly used methods include 2,2-diphenyl-1-picryhydrazyl (DPPH assay), Ferri reducing/antioxidant power assay (FRAP assay), oxygen radical absorbance capacity (ORAC) and 2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid) (ABTS assay) [6, 10]. A single assay could not accurately reflect the antioxidant activity in a complex system due to multiple reaction characteristics. Consequently, at least two methods should be used in order to fully elucidate the antioxidant activity of fruit.

Flavonoids and phenolic compounds play important roles in the antioxidant activity in fruit. There are more than 3000 literatures published on phenolic compounds in the last two decades [6]. However, few literatures focused on tropical and subtropical fruit. It has been found that antioxidant activity of honey pineapple, banana and Thai seedless guava pulp has been compared and found that Thai seedless guava pulp had the highest antioxidant activity among the three fruit [9]. Guava fruit is popular for its high content in phenolic compounds, vitamin C, and carotenoids. However, limited literatures published focused on the antioxidant activity of guava fruit and leaf extract [11-14]. Due to paucity of information about the antioxidant activity of guava is limitation to utilize guava fruit. Genetic/cultivars and harvest time strongly influence the antioxidant activity [15]. In the present study, we compared the changes of fruit color and antioxidant activity in red-fleshed guava fruit at different maturation stages. The main aim of this study was to analyze the correlations between the antioxidant activity and the total phenolics and total flavonoids in guava fruit.

2. MATERIALS AND METHODS

2.1. Plant materials and sampling

Guava fruit of ‘Sijitao’ (*Psidium guajava* L.) with different flesh colour were harvested at different mature stages from a commercial orchard in Lianjiang city, Guangdong Province, South China. After harvest, the fruit were immediately transported to the key laboratory of

tropical crop product processing on the day of harvest. Red-fleshed guava fruit were harvested at three different maturity stages, namely ‘immature’, ‘mature’ and ‘ripe’ based on the fruit colour. Uniform, healthy, disease and bruise free fruit were selected as the experimental materials. The maturity of guava fruit was determined by the conventional indices, such as peel colour, fruit firmness and fruit aroma. Thirty fruits were chosen randomly at each mature stage for chemical analysis, and flesh was sliced from guava fruit into liquid nitrogen, and then stored at -40°C until further analysis.

2.2. Assessment of skin color and flesh color

Colour of the skin and flesh of guava fruit was measured with a colorimeter (colour i5, X-Rite, U.S.A), which provided CIE L*, a*, b*, and C values. Negative a* value indicate green colour, while positive indicate red. A higher positive b* value indicate yellow colour, and negative indicate blue. The value of C, namely chroma, indicates the intensity or purity of the colour. The values of a* and b*can be converted to hue angle degree (ho=arctan [b*/a*]), which 0oindicates red, 90° yellow, 180° bluish-green, and 270 blue [39]. Measurements were made on ten fruits for each sample.

2.3. Sample extraction

Each sample of frozen fruit tissue (2 g) was ground by hand to fine powder in liquid nitrogen and then transferred to a 10-mL tube with 5 ml 80% ethanol solution containing 0.1% of hydrochloric acid. The mixture sonicated for 30 min at room temperature (about 25°C), and then centrifuged at 5 000g for 5min. The supernatant was removed to a new tube and used for analysis of phenolics, flavonoids, and the estimation of antioxidant capacity. All of the analysis and measurements were carried out in triplicate.

2.4. Measurement of total phenolics contents

Total soluble phenolics content was measured by the Folin-Ciocalteau reagent method with some modification [40]. Briefly, an aliquot of 100 µl extracted solution of each sample was mixed with 4.0 ml distilled water, 0.5 ml Folin-Ciocalteau reagent, and 1.0 ml of2 g/100 ml sodium carbonate was added. The mixture incubated for 2 hour at room temperature. The absorbance of the mixture was determined by a UV-visible spectrophometer (SP-752N, Shanghai Spectrum Instrument Co., LID, China) at 750 nm. A calibration curve was made using a standard solution of Gallic acid ($y=0.071x-0.043$, $R^2=0.997$). Results were expressed as mg Gallic acid equivalent per 100 g FW of sample.

99 **2.5. Determination of total flavonoids**

100 The concentration of total flavonoids was measured by a colorimetric assay with slight
101 modification [41]. The solution (0.5 ml) extracted by 80% ethanol diluted with 1.5 ml
102 methanol and 0.3 mL of NaNO2 was added. After 6 minutes of reaction, 0.3ml 10%
103 Al(NO3)3,2 ml 2M NaOH and 4.9ml ethanol solution were added to the tube. The solution
104 was mixed and measured the absorbance after 10 min against a blank at 510 nm using a
105 UV-visible spectrophometer (SP-752N, Shanghai Spectrum Instrument Co., LID, China).The
106 amount of total flavonoids was calculated as Catechuic acid (Sigma Chemical, St. Louis,
107 USA) equivalents from a calibration curve of standard solutions, and expressed as milligram
108 of Catechuic acid/ 100 g of fresh weight.

109 **2.6. DPPH free radical scavenging assay**

110 The free radical scavenging activity of fruit extracts was measured according to DPPH
111 method [42]. A 100μl aliquot of fruit extract was mixed with 3.9ml of 60μM DPPH methanolic
112 solution. The reaction for scavenging DPPH radicals was kept in the dark at room
113 temperature for 6 hours. The absorbance was measured with a UV-visible spectrophometer
114 (SP-752N, Shanghai Spectrum Instrument Co., LID, China) at 515 nm, against a blank of
115 methanol without DPPH. Results were expressed as percentage of inhibition of the DPPH
116 radical. Percentage of inhibition of the DPPH radical was calculated according to the
117 equation [(Abs control-Abs sample) ×100/Abs control]. Trolox was used as the standard.
118 Results were expressed as mmol Trolox equivalents/100 g fresh weight.

119 **2.7. Ferric reducing/antioxidant power assay (FRAP assay)**

120 The FRAP assay was performed with slight modification according to the method by Guo
121 [43]. FRAP reagent was made from 1mM TPTZ, and 2 mM ferric chloride in 300 mM sodium
122 acetate buffer (pH 3.6). A 200μl aliquot of fruit extract was mixed with 1.8ml FRAP reagent,
123 and absorbance was measured after a 10 mins reaction using a UV-visible spectrophometer
124 (SP-752N, Shanghai Spectrum Instrument Co., LID, China) at 595 nm against a blank of
125 methanol. The antioxidant capacity of the fruit extract was determined by its ability to reduce
126 ferric to ferrous ions in the FRAP reagent. The standard curve was constructed using
127 concentration of Trolox from 0 to 1000 μM and the linear range was used for calculation.
128 FRAP assay results were expressed on a fresh weight basis as mmol Trolox equivalents/100
129 g fresh weight.
130

2.8. Statistical Analysis

Data were analyzed using Data Processing System (DPS) version 3.01 (Zhejiang University, Hangzhou, China). Standard errors (SEs) were calculated. Values were expressed as means ± standard deviations. Differences were considered significant at $P < 0.05$. All the analyses were carried out in triplicates.

3. RESULTS

3.1. The changes of fruit color in guava fruit with different cultivar and maturity

The colour changes between different maturation stages were obviously observed in red-flesh guava according to the colour index values (Table 1). The *L** values was lower in immature fruit peel than mature fruit, whereas the mature fruit showed a lower *L** value in fruit flesh. The increasing values of *L** indicated that the skin of red-fleshed guava became lighter and smoother during fruit maturation. Both values of C and h^o of fruit peel decreased during the period of fruit maturation. The lower h^o value indicated the fruit changed to yellow (h^o=79.00) from green (h^o=105.44). There was no obvious difference in C values of fruit flesh between different maturities. There was a dramatically decrease in h^o values in fruit flesh, from 101.70 to 42.69. That was due to the changes of flesh from white to pink (Table 1).

There were obvious differences in colour index values between red-fleshed and white-fleshed guava except for L value in fruit peel (Table 1). The white-fleshed guava had a lighter flesh than red-flesh guava, according to the higher L value (73.81). From visual observation, the difference was not obvious between red-fleshed and white-fleshed guava. But the colour index values of fruit peel differed from each other apparently (Table 1). The white-fleshed guava showed a greenish yellow (h^o=100.65), whereas the red-flesh had a reddish yellow skin when the fruit ripen (h^o=79.00). The red-fleshed guava showed a red flesh (h^o=42.69), and the white-fleshed had an ‘opaque’ yellow (h^o=85.87).

Table1. Difference in flesh color of different guava cultivars and maturity

Parameters		Peel	Flesh
L	HR	57.43±5.72	73.02±2.50
	NR	68.49±4.30	72.03±0.92
	RP	70.96±2.95	57.81±2.94
	WG	73.05±2.31	73.81±3.76
c	HR	47.35±1.04	43.14±1.83
	NR	22.91±4.91	28.84±0.80
	RP	15.93±1.27	34.20±3.62
	WG	42.01±2.83	10.64±2.73
h	HR	105.44±3.28	101.70±1.48
	NR	82.52±4.25	98.31±2.75
	RP	79.00±4.33	42.69±4.13
	WG	100.65±1.32	85.87±7.09

Note, HR, half ripe of red-fleshed guava fruit; NR, near ripe of red-fleshed guava fruit; RP, ripe fruit of red-fleshed guava fruit; WG, ripe fruit of white-fleshed guava fruit

2.2 Comparison of total phenolics, flavonoids of different guava cultivars and maturity

The contents of total phenolics and total flavonoids differed significantly during maturation of red-flesh guava. The ripe fruit had the highest content of total phenolics and flavonoids (987.74 and 438.51), while the half ripe had the lowest (147.79 and 113.57). The content of total phenolics and total flavonoids increased during the ripening of guava fruit, and the total flavonoids content near to ripening of guava fruit was similar to ripened fruit. The content of total phenolics in ripe guava was higher than in half ripe and near ripe fruit. It was obvious that the contents of total phenolics and total flavonoids in ripe red-fleshed guava were higher than white-fleshed guava (Table 2). The content of total flavonoids in ripe white-fleshed guava was lower than in red-fleshed guava, 205.94 and 438.51 mg $100g^{-1}$, respectively. Similarly, the content of total phenolics of white-fleshed guava was one fold lower than red-fleshed guava.

Table2. Content comparison of total phenolics, flavonoids of different guava cultivars and maturity

	Total flavonoids (mg GAE/100g fresh weight)	Total phenolics (mg GAE/100g fresh weight)	DPPH	FRAP
HR	113.57±1.92	147.79±18.73	303.09±10.60	83.75±10.83
NR	433.62±26.07	788.22±36.84	846.60±2.31	104.92±20.08
RP	438.51±38.45	987.74±22.80	831.91±19.26	116.92±13.82
WG	205.94±22.26	391.84±18.50	732.64±25.94	162.08±2.75

Note, HR, half ripe of red-fleshed guava fruit; NR, near ripe of red-fleshed guava fruit; RP, ripe fruit of red-fleshed guava fruit; WG, ripe fruit of white-fleshed guava fruit

2.3 Comparison of antioxidant capability of different guava cultivars and maturity

There are many antioxidants present in various fruits, and the measurements of antioxidant capability of different fruits should be confirmed at least with two methods. Several protocols have been made to estimate the antioxidant capability of different plant material. DPPH free radical-scavenging assay and ferric reducing/antioxidant power assay (FRAP assay) were used to study the antioxidant capability of the guava fruit. The antioxidant capability of guava fruit varied in different cultivars and during maturation. It was found that antioxidant capability of red-fleshed guava increased in both methods. The ripe fruit of red-fleshed guava had the highest antioxidant capability, while the half-ripe fruit had the lowest. As for different cultivars, the red-fleshed guava showed a higher antioxidant capability than white-fleshed, despite the different reaction mechanisms of DPPH and FRAP.

2.4. Correlation between antioxidant ability and content of total flavonoids, phenolics

The correlation coefficients between total flavonoid, total phenolics and antioxidant capability varied from 0.67 to 0.98 (Table 3). Highly positive correlations were observed between total flavonoid and total phenolics (r^2=0.98). Weak correlations were found between total flavonoid and FRAP (r^2=0.67). There were strong correlations between total flavonoid, total phenolics and antioxidant capability in most cases (r^2>0.98), except relationship between total flavonoid and FRAP (r^2=0.67).

Table 3. Correlation coefficients between total flavonoid, total phenolics and antioxidant capability

	Total flavonoids	Total phenolics	DPPH	FRAP
Total flavonoids	1			
Total phenolics	0.98	1		
DPPH	0.87	0.87	1	
FRAP	0.67	0.80	0.72	1

3. DISCUSSION

Guava is an exotic and the most valuable fruit of the *Myrtaceae* family in tropical and subtropical regions [16, 17]. It is widely distributed and cultivated in many tropical and subtropical countries, mainly in India, China, South Africa, and Florida [17]. Guava fruit is commercially important and popular due to its intense aroma, rich nutritional and medicinal value. The content of Vitamin C in an apple guava is over four times than a single orange [18]. Guava can be divided into two kinds, namely white-fleshed and red-fleshed. The

external skin color changed from light green or yellow from green during ripening, regardless of the flesh color. It was found that the skin color changed from green to yellow in red-fleshed guava, while the white-fleshed guava showed a greener skin color (Table 1). The degreening of fruit skin may due to the degradation of chlorophyll, and different rate of chlorophyll degradation led to the different skin color indifferent guava cultivars. Red-fleshed guava pulp became to pink during ripening, while it was yellow in white-fleshed guava. Lycopene accumulated during maturation and peaked when red-fleshed fruit ripe, while it was not found in white-fleshed guava [19]. The mechanism of color change in guava fruit is different from apple and pear. In the most fruit, the coloration of fruit peel or pulp due to the accumulation of anthocyanin [20].

Phenolics, mainly included by flavonoids, phenolic acids, stilbenes, coumarins and tannins are, are one of the main secondary metabolic products. These are well-known due to its potent antioxidant activities and to diffuse the free radicals [6, 21]. In addition to rich in vitamins and dietary fiber, a variety of phenolics are also present in guava fruit as antioxidant [14]. There was significant variation in the content of total phenolics and total flavonoids in different maturation stages and cultivars (Table 2). The ripe red-fleshed guava showed the highest content of total phenolics and total flavonoids, 987.74 and 438.51 mg $100g^{-1}$, respectively. The content of bioactive compounds such as total phenolics flavonoids, and also anthocyanins increased during fruit maturation and peaked during ripening in apple [22], grape [23] and bayberry [24].

The phenolics content in fruit not only affected by variety, ripeness, maturity, and environmental factors [6], as well as species also play an important role on the total phenolic content [25]. Previous studies have indicated the phenolics have strong antioxidant properties. The total phenolics content of ripe guava wasF found to be higher than unripe fruit (Table 2). Total phenolics content of fully-ripen guava was lower than unripe guava in Pakistan [26]. These differences might be due to different cultivars, environmental factors, and different extraction solvents in sample preparation. Different genotypes had different change trends of total phenolics during fruit maturation [27]. High contents of total phenolics in pomegranates husk showed to be more tolerant to scald [28]. Flavonoids account for about two thirds of the dietary phenols and they have strong by interfering with the formation and propagation reaction of free radicals, chelate the transition metals, or inhibit the enzymes involved in the initiation reaction [29,30]. A similar increasing trend with guava fruit maturation was also found in total flavonoids content. Ripe fruit showed the highest content of total flavonoids. Similar results were found in crabapple [25], table grape [30] and blue berry [31].

The DPPH assay and FRAP assay are two of the most popular methods used to evaluate antioxidant activity in fruit. Regardless of fruit maturation and cultivars, both DPPH and FRAP showed red-fleshed guava had the highest antioxidant activity at fully ripen stage. And with the maturation of guava fruit, the increase in scavenging capacity might be related to increase of total phenolics (Table2). In agreement to our finding, higher DPPH scavenging activity of guava fruit might be associated to its higher content of total phenolics [32]. It was

observed that red-fleshed guava had a higher antioxidant activity than white-fleshed. Guava fruit had the highest antioxidant activity among guava, mango, papaya, and lemon [33]. Flors *et al*. reported that the antioxidant activity in pink-pulp guava fruits was higher than in white pulp cultivars [34]. Fruit with dark color showed a higher antioxidant activity such as in apple [22], bayberry [24], and mulberry [35].

Correlation was applied to investigate the relationship between total phenolic, total flavonoids and antioxidant activity in guava fruit and significantly positive correlations were found, with the exception of total flavonoid and FRAP (r^2=0.67) (Table 3). Our results are in accordance with previous studies in sweet berry [36], Chinese dwarf cherry [37], crabapple [25], and pomegranate [38] which showed the strong correlations between the total phenolics content and antioxidant activity. These findings indicated that higher antioxidant activity is mainly due to the accumulation of total phenolics and flavonoids in guava fruit.

4. CONCLUSION

The results of the present study showed that the contents of total phenolics and flavonoids increased during maturation of guava fruit. It was also found that red-fleshed guava fruit not only had higher contents of total phenolics and flavonoids, and also showed higher antioxidant activity with DPPH and FRAP methods than white-fleshed guava. Both DPPH and FRAP indicated that antioxidant activity increased during fruit maturation. Strong and positive correlations were found between total phenolics, total flavonoids and antioxidant activity. It could be suggested that guava fruit is potent free radical scavengers and may be utilized as a good source of natural antioxidant.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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