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Quantification of bioactive compounds in guava at different ripening stages

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Abstract

Guava (*Psidium guajava* L.) is one of the most important tropical fruits belonging to the genus *Psidium* and the *Myrtaceae* family and claim to have phenolic compounds that have been reported to possess strong antioxidant activity. This study was aimed to evaluate the bioactive constituents in guava cultivars at different ripening stages by HPLC. The five guava cultivars were selected at different ripening stages and the bioactive components were analysed by high-pressure liquid chromatography. The quantification of bioactive compounds revealed that the highest amount of bioactive compounds was found in cultivar Safeda at the unripe stage, while a minimum amount was found in ripe Apple Colour guava cultivar. The six bioactive compounds were quantified in the range of gallic acid (9.46-63.08 mg/100 g), quercetin (0.11-2.51 mg/100 g), myrcetin (0.09-0.034 mg/100 g), ascorbic acid (7.45-75.07 mg/100 g), apegenin (0.01-0.032 mg/100 g) and lycopene (0.34-0.92 mg/100 g). The exploratory evaluation of guava samples was performed through Principal Component Analysis (PCA), the bioactive compounds, lycopene, myricetin, and quercetin are dominant variables on this PC1 (61.52%) (Scores better than 0.7), thereby causing greater variability among these samples. The second principal component (PC2) represents 16.54% of the total variance and the ascorbic acid, gallic acid and apeginin (score better than 0.7), are the dominant variables on this PC.

1. Introduction

It is known that plants are a rich source of secondary metabolites like flavonoids, carotenoids, alkaloids, terpenoids and tannins and they have been implicated in several therapeutic methodologies. *Psidium guajava* L. is a small tree native to Central America; it is popularly known as guava and belongs to the myrtle family (*Myrtaceae*). The guava tree has been distributed through many countries as a result of its capacity to grow in tropical and subtropical conditions (Morton, 1987). Compounds such as carotenoids and flavonoids have already been recognised as either inhibitor of oxidative stress or compounds that reduce oxidative stress (Sies and Stahl, 1995) because they can act with antioxidants that are able to inactivate free radicals and their action (Halliwell, 1996; Devasagayam *et al.,* 2004). The flavonoids, known as a natural antioxidant, are the main bioactive compounds found in fruits (Robbins, 2003; Lampila *et al.,* 2009). Carotenoids are abundant in fruits and vegetables but are usually masked by green-coloured chlorophyll. The degradation of chlorophyll during maturation enables the carotenoids to be visualized, for example, in dying leaves during autumn and matured fruits (tomatoes, oranges, grapefruit) (Rabinowitch *et al.,* 1982). The key nutritional role of carotenoids is provitamin A, which is commonly known to improve vision. Additionally, carotenoids are known to act as an antioxidant in the human body (Perera and Yen, 2007; Rao and Ali, 2007). High-performance liquid chromatography (HPLC) is currently the preferred procedure for carotenoid and flavonoid analysis. HPLC columns offer high resolving power and numerous papers have been published on the HPLC separation of carotenoids (Rodriguez-Amaya, 2015). Only a few of these papers included quantification, however, and discrepancies continue to exist in the reported quantitative data.

The nutritional and health-promoting properties of guava, together with the increased interest in its antioxidant properties, indicate the potential nutraceutical use of this fruit (Ho *et al.*, 2012). Therefore, there is a need for the proper selection of cultivars with the appropriate bioactive composition for the intended use of the fruit. This study focuses on the

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isolation of different bioactive compounds from five guava cultivars fruit extract at different ripening stages.

2. Materials and methods

2.1 Plant materials

A total of five guava cultivars namely, Lalit, Allahabad Surkha, Allahabad Safeda, Chittidar and Apple colour were collected at different ripening stages from an orchard in Khusroobagh (under the Department of Horticulture and Food Processing, Uttar Pradesh Government), Allahabad and stored at 15±2°C with a relative humidity of 90-95%.

2.2 Sample preparation

Guava was weighed at 1 g (triplicate, n=3) and extracted (1:50, w/v) in 10% dimethylsulphoxide in 90% methanol (3×50 mL, 4 hr each) and the supernatants were obtained by centrifugation at 5000 rpm for 20 mins. Phenolic acids were separated by ethyl acetate phase separation (4×50 mL) and the fractions were pooled (Subba Rao and Murlikrishna, 2002).

2.3 Estimation of Polyphenols and antioxidant analysis

Fruit extract of guava cultivars along with standard solutions was prepared and filtered through a 0.22 µm filter (Milli-pore, Billerica, USA). Samples were analysed on analytical HPLC (Metrohm Technologies) fitted with an automatic degasser, C-18 column 4.6×250 mm and a Photo-diode array detector (DAD). A mobile phase of different organic solvents acidified methanol: water (50:50), petroleum ether: water (10:90), ethanol: water (10:90) and acetonitrile: water (10:90 v/v) was degassed and filtered through a 0.22 µm filter paper. The detector was set in scan mode, 210-550 nm during the analysis, while the flow rate was set at 1 mL/min. A sample volume of 20 µL was injected into the column. The polyphenolic and antioxidant content of extract were measured by comparing peak retention time and area under the chromatographic peak of standard solution.

2.4 Statistical analysis

All the experiments were performed in triplicates and mean were reported. Multivariate technique Principal Component Analysis (PCA) was applied for a better understanding of the characteristics results obtained by different analyses.

3. Results and discussion

3.1 Isolation of antioxidants from guava cultivars

In the present study, bioactive compounds of guava fruit at different ripening stages have been investigated by different extraction procedures. The comparative efficiency of the solvents was estimated from the total peak areas of the HPLC chromatograms obtained. The maximum extraction of the bioactive compound was observed in acetonitrile: water (10:90 v/v), which was proved to be a most efficient combination for polyphenol (gallic acid), three flavonoids (myrcetin, apigenin and quercetin), ascorbic acid and carotenoid (lycopene) present in all the samples.

3.2 Quantification of phenolic acid

Phenolic compounds in fruits have received significant attention in recent years due to their potent antioxidant capacities and their ability to reduce the risk of diseases caused by oxidative stress, such as cancer (Kubola and Siriamornpun, 2011; Deng *et al.*, 2013; de Carvalho-Silva *et al.*, 2014; Siriamornpun *et al.*, 2015). In the present study, gallic acid was identified and quantified in the green and ripe guava fruits. The results showed that the content and composition of gallic acid was significantly different ($p<0.05$) between the cultivars and the maturity of the fruits. The gallic acid clearly decreased with ripening stages in all the selected guava cultivars.

The unripe stage of Lalit guava had the major amount of gallic acid (63.08 mg/100 g) among all the cultivars. The least amount of Gallic acid was seen in the ripe Lalit guava (9.46 mg/100 g). The semi-ripe variety, however, had a moderate level of gallic acid (25.09 mg/100 g). The level of Gallic acid was therefore found to be more in the unripe stage as compared to the other two stages in the cultivars. The semi-ripe and ripe stages of Surkha and Safeda had a gallic acid level of 31.73 mg/100 g; 11.30 mg/100 g; and 24.18 mg/100 g, respectively. The gallic acid of semi-ripe and ripe varieties of Chittidar cultivar was 22.18 mg/100 and 10.09 mg/100 g, respectively. The Gallic acid content of unripe Chittidar guava samples had nearly double that of the semi-ripe Safeda cultivar (i.e., 49.08 mg/100 g). The unripe stage of Apple colour had the second-highest amount of gallic acid among the five cultivars (60.11 mg/100 g). The semi-ripe and ripe variety showed a gallic acid level of 28.87 mg/100 g and 12.54 mg/100 g, respectively. The amount of gallic acid in the unripe fruit of all fruits investigated was higher than that of the ripe fruit. Similar results were reported by Siriamornpun and Kaewseejan (2017). The maximum decrease in gallic acid content (85.03%) was found in cultivar Lalit, while Apple colour showed a decrease of 79.14% during the ripening of fruits.

Kondo *et al.* (2005) also detected gallic acid, catechin, epicatechin and chlorogenic acid in guava, out

of all these compounds catechin found in the highest concentration (45 µmol/kg of fresh weight). However, Freda *et al.* (2017) found 90.90 mg/100 g of gallic acid was present in red guava pulp, 79.80 mg/100 g in conventional sweet guava paste and 82.60 mg/100 g gallic acid in light sweet guava paste.

3.3 Quantification of flavonoids

Flavonoids are one of the most important polyphenolic compounds with human health benefits due to their potent antioxidant and pharmacological effects (Miean and Mohamed, 2001; Khanam *et al.*, 2012). In our study, three flavonoids, namely myrcetin, apigenin and quercetin were identified and quantified in the unripe semi-ripe and ripe fruits from the five fruits. According to Siriamornpun and Kaewseejan (2017), flavonoids were significantly different depending on cultivar and ripeness of the fruit. The differences in the amount of flavonoids may be due to the genetic variability and the ripening leading to variations in the biosynthesis of flavonoids in these fruits.

The highest myrcetin was found in the ripe Surkha (0.34 mg/100 g) and the lowest count was found in the unripe stage of the same variety (0.011 mg/100 g). Similar patterns were seen with cultivar Apple colour; (0.028 mg/100 g; 0.015 mg/100 g and 0.11 mg/100 g), respectively in unripe, semi-ripe and ripe stages of maturity. While other cultivars showed an opposite pattern as the content of myrcetin increases with maturity (Figure 1). The unripe, semi-ripe and ripe stages of Lalit cultivar had increased Myrcetin levels with maturity viz. (0.025 mg/100 g), (0.034 mg/100 g) and (0.042 mg/100 g); Chittidar (0.031 mg/100 g; 0.016 mg/100 g; and 0.09 mg/100 g), respectively. A similar pattern has been observed in Safeda cultivar (Table 1). The maximum increase in myrcetin acid content (292.86%) was found in cultivar Apple colour, while Lalit showed an increase of 68.00% during the ripening of fruits. Musa *et al.* (2015) suggested the levels of myricetin in Sungkai guava fruit, flesh, and skin were 80.38, 93.75, and 51.60 mg/kg, respectively, and for Semenyih guava, the levels were 83.05, 84.00, and 73.75 mg/kg for fruit, flesh, and skin, respectively.

The flavonoid that was found to be in the least amount was apigenin, besides being absent in the unripe stage of all the selected cultivars. Among the cultivars where Apigenin was found, the semi-ripe variety of Lalit Guava had the least amount (0.01 mg/100 g) of flavonoid as compared to the ripe variety (0.025 mg/100 g). The apiginine content in semi-ripe stage and ripe stage of Surkha was 0.015 mg/100 g and 0.029 mg/100 g, respectively. The semi-ripe and ripe stages of Safeda and Chittidar had apigenin levels of 0.009 mg/100 g, 0.015 mg/100 g, 0.022 mg/100 g and 0.012 mg/100 g, respectively. Apigenin was also absent in the semi-ripe stage of Apple colour but was found in the ripe stage (0.023 mg/100 g). Apegenin was not detected in all fractions from both sungkai and semenyih guava (pink guava) as suggested by Musa *et al.* (2015), which is in agreement with the present work. While Miean and Mohamed (2001) reported guava contains 550 and 579 mg/kg of the dry weight of the flavonols myricetin and apigenin, respectively. The maximum increase in apigenin content (275.00%) was found in cultivar Safeda, while Lalit showed an increase of 93.33% during the ripening of fruits, but cultivar Chittidar showed a 45.45% decrease in apigenin content during the ripening from semi-ripe to ripe.

Table 1. Bioactive compounds of guava cultivars at different ripening stages

Cultivars		Compounds					
Ripening stages		Gallic acid (mg/100 g)	Myrcetin (mg/100 g)	Apegenin (mg/100 g)	Quercetin (mg/100 g)	Ascorbic acid (mg/100 g)	Lycopene (µg/g)
Lalit	Unripe	63.08±0.025	0.025±0.003	-	2.43±0.01	9.11±0.30	-
	Semiripe	25.09±0.03	0.034±0.001	0.01±0.001	1.02±0.02	19.09±0.13	-
	Ripe	9.46±0.02	0.042±0.002	0.025±0.004	0.98±0.03	35.00±0.21	9.00±1.7
Surkha	Unripe	58.8±0.023	0.11±0.07	-	2.51±0.03	11.28±0.14	-
	Semiripe	31.73±0.090	0.23±0.067	0.015±.007	0.34±0.01	28.11±0.12	-
	Ripe	11.30±0.035	0.34±0.054	0.029±0.003	0.11±0.03	49.34±0.23	8.6±1.03
Safeda	Unripe	59.11±0.055	0.016±0.001	0.004±0.001	3.22±0.05	48.09±0.13	-
	Semiripe	24.18±0.053	0.027±0.01	0.009±0.0015	1.02±0.04	56.10±0.17	-
	Ripe	11.78±0.055	0.037±0.034	0.015±0.003	0.89±0.03	75.07±0.26	-
Chittidar	Unripe	49.08±0.06	0.031±0.00	-	1.23±0.02	10.22±0.46	-
	Semiripe	22.18±0.03	0.016±0.03	0.022±0.04	0.93±0.07	22.45±0.35	-
	Ripe	10.09±0.06	0.09±0.002	0.012±0.01	0.56±0.02	38.90±0.23	3.7±0.09
Apple colour	Unripe	60.11±0.07	0.028±0.001	-	2.54±0.01	7.45±0.22	-
	Semiripe	28.87±0.06	0.015±0.03	-	1.72±0.08	18.90±0.11	9.4±2.01
	Ripe	12.54±0.06	0.11±0.01	0.023±0.01	0.44±0.02	26.04±0.33	33.2±4.13

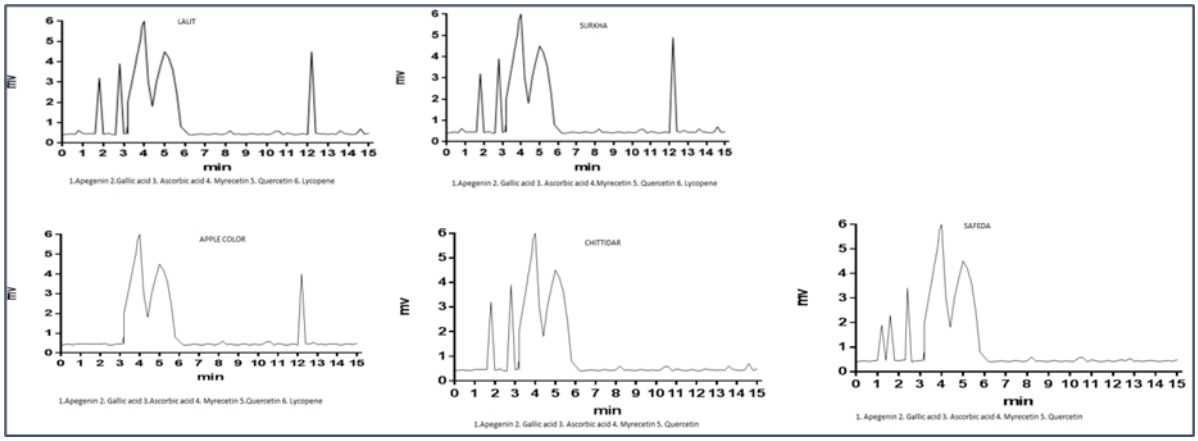

Figure 1. HPLC chromatogram of fully ripened guava cultivars Lalit, Surkha, Apple Colour, Chittidar and Safeda using C-18 column eluted with methanol: acetonitrile as mobile phase.

The quercetin was the major flavonoid present in the fruit, and the highest amount was found in Unripe Safeda (3.22 mg/100 g) and the lowest in the ripe stage of Surkha (0.11 mg/100 g). The level of Quercetin was decreased with maturity in all three stages. The unripe, semi-ripe and ripe stages of Lalit guava had the level of 2.43 mg/100 g, 1.02 mg/100 g and 0.98 mg/100 g of flavonoid, respectively (Table 1). Surkha cultivar showed levels of 2.51 mg/100 g and 0.34 mg/100 g of flavonoid in unripe and semi-ripe stages, respectively and a similar trend was followed by Safeda and Chittidar cultivars. Apple colour also showed decreased quercetin level along with the stages of maturity *viz*. 2.54 mg/100 g in unripe, 1.72 mg/100 g in semi-ripe and 0.44 mg/100 g in the ripe stage. The maximum decrease in quercetin content (95.62%) was found in cultivar Surkha, while Chittidar showed a decrease of 54.47% during the ripening of fruits. The concentration of phenolic bioactive compounds in fruits depends on the degree of maturity, variety, climate, soil composition, geographic location, and storage conditions, and other factors, which explains the discrepancy between data from different articles, for the same type of sample (Das Santos *et al.*, 2017). In the previous study on fresh and processed Brazilian fruits by Hoffmann – Ribani *et al.* (2009), the concentration of quercetin in guava (1.30 mg/100 g) is compatible with the values found in this study. In the current study maximum quercetin was found in unripe Safeda cultivar which is similar to results reported by Das Santos *et al.* (2017), that is high quercetin content was found in white and green guava cultivars as compared to others.

3.4 Quantification of ascorbic acid

In addition to being the biologically active form of vitamin C, is the most commonly found and widely distributed in products of plant origin. It is primarily in citrus fruits and leafy vegetables. They are characterized as poor sources of vitamin C. The vitamin content of the fruit can vary, depending on the species, maturity stage, genetic variants, postharvest handling, storage conditions, and processing. The content and stability of these nutrients in fresh food can influence its nutritional quality (Szeto *et al.*, 2002). The highest amount of ascorbic acid was found in the ripe Safeda guavas (75.07 mg/100 g) and the lowest in the unripe stage of Apple colour guava (7.45 mg/100 g). The Lalit guava showed a moderate amount of ascorbic acid with the least in the unripe stage (9.11 mg/100 g). The ascorbic acid levels in Surkha were 11.28 mg/100 g, 28.11 mg/100 g and 49.34 mg/100 g respectively in the unripe, semi-ripe and ripe stages. Chittidar showed the level of ascorbic acid in unripe, semi-ripe and ripe stages were 10.22 mg/100 g, 22.45 mg/100 g and 38.90 mg/100 g, respectively. Levels of ascorbic acid seen in the Apple colour cultivar were 18.90 mg/100 g and 26.04 mg/100 g in the semi-ripe and ripe stages respectively (Table 1). Hence, a considerable increment of the ascorbic acid level was seen in all the five cultivars with maturity, from unripe, semi-ripe, till the ripe stages of each cultivar. The maximum decrease in quercetin content (337.41%) was found in cultivar Surkha, while Chittidar showed a decrease of 56.10% during the ripening of fruits.

Mitra *et al.* (1983) reported that Vitamin C content was highest in Lucknow-49 and Apple Colour, whereas Pear Shaped, Red Fleshed and Harijha varieties had poor Vitamin C content. According to Singh *et al.* (1984), Guava varieties Anakapalli, Kothrud, Lucknow-49, Portugal and Seedless contained high ascorbic acid content (201.20 mg/100 g to 225.70 mg/100 g), while cultivars. Chittidar (84.00 mg/100 g) had a minimum amount of ascorbic acid. Chauhan *et al.* (1986) observed that ascorbic acid content showed considerable fluctuation during different seasons and it was highest in cultivar Allahabad Safeda (329.28 mg/100 g) during the rainy season. Thuaytong and Anprung (2011) also reported the ascorbic acid content in white and red guava fruit up to 130.00 and 112.00 mg/100 g, respectively which is higher than the present study.

3.5 Quantification of lycopene

Lycopene could only be detected in the ripened stage of the cultivar (with the highest amount in the apple colour cultivar i.e., 0.92 mg/100 g); except being present in the semi-ripe stage of Apple colour variety (lowest, 0.34 mg/100 g). It was absent throughout the ripening in cultivar Safeda (Table 1). Moreover, a considerable amount of Lycopene was seen in the ripe stages of two cultivars Lalit (0.90 mg/100 g) and Surkha (0.86 mg/100 g). Lycopene extraction from *Psidium guajava* L. carried out by Fabienne Priam *et al.* (2017) suggested that the lycopene concentration obtained was 2.11 mg/100 g in guava which is higher than the findings of the current study. Rani *et al.* (2017) also reported a higher amount of lycopene in red guava fruit i.e., 52.16 mg/kg. The pink/ red flesh colour found in some varieties of guava has been attributed to the presence of lycopene. Poher *et al.* (2003) reported lycopene content as 4.5 to 5.5 mg/100 g in Red fleshed guava variety. Ordóñez-Santos and Vázquez-Riascos (2010) carried out a study on pink guava fruit and found the lycopene content of the fresh fruit to be 3.55 mg/100 g. However, Boora (2012) observed a higher amount (7.45 mg/100 g) of lycopene content in fresh Punjab Pink variety of guava. Kumar (2015) found that the lycopene content of fresh fruits of Punjab Pink was 3.74 mg/100 g. Wilberg and Rodriguez-Amaya (1995) reported lycopene content of about 5.34 mg/100 g in guava fruit which is similar to the present study.

Based on current findings, it has been demonstrated that unripe fruits of all cultivars studied could serve as potential sources of bioactive compounds, especially phenolics and flavonoids. Thus, this information may be useful for consumers who need to choose fruits that provide the highest specific bioactive compounds and health benefits. Moreover, it is considered that the profile of bioactive compounds could be a potential metabolite marker to determine the ripening stages of the three fruits studied as the fruit undergoes the ripening the ascorbic acid content decreases while the amount of lycopene increases (Table 1).

3.6 Principal component analysis (PCA)

An exploratory evaluation involving five guava cultivars was performed using Principal Component Analysis (PCA), comprising six variables: gallic acid, quercetin, apigenin, myricetin, ascorbic acid, and lycopene (mg/100 g) by HPLC. PCA analysis was applied, after auto-scaled, using the Software IBM SPSS Statistics 20. Components (PC1×PC2) describe 78.06% of the total variance of the data and provide discriminatory information related to the samples. Figure 2 shows the score plots (PC1×PC2) of the principal

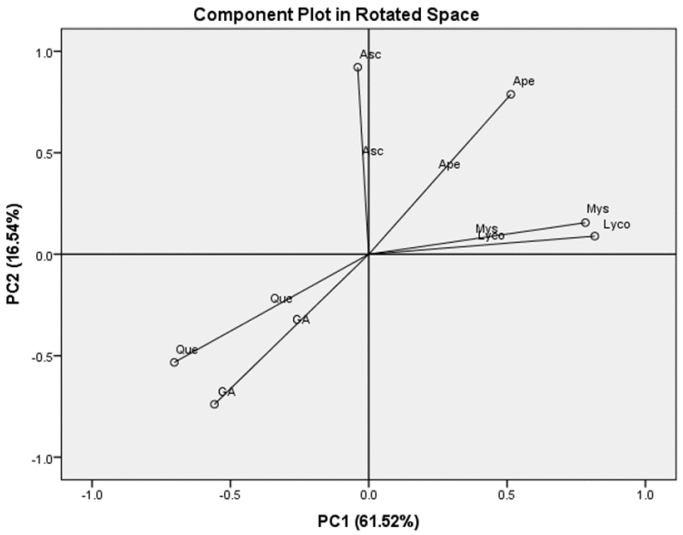

Figure 2. Principal component analyses for 5 guava cultivars (*Psidium gujava* sp.) and the eigenvector circle of the variable: myrcetin (Mys), Quererecetin (Que), apeginin (Ape), Lycopene (Lyco) and ascorbic acid (Asc)

component analysis (PCA) at different ripening stages of guava (*Psidium guajava* L.) in relation to bioactive components (mg/100 g) by HPLC. The eingen values of the correlation matrix for PC1, and PC2, were, respectively: 3.69 and 0.99. The first principal component (PC1) describes 61.52% of the total variance. The bioactive compounds: lycopene, myricetin, and quercetin are the dominant variables on this PC (scores better than 0.7), thereby causing greater variability among these samples. The second principal component (PC2) represents 16.54% of the total variance and the ascorbic acid, gallic acid and apeginin (score better than 0.7), are the dominant variables on this PC. Similarly, Dantas *et al.* (2013), Dos Santos *et al.* (2017), had classified the guava fruits according to their maturity stages by conducting PCA analysis.

4. Conclusion

This study was performed to determine the levels of gallic acid, ascorbic acid, quercetin, apigenin, myricetin and lycopene between ripening stages and cultivars. The results indicate that guava is a rich source of polyphenols, ascorbic acid and flavonoids and lycopene and also affected by the type of cultivar and their maturity stages. The lycopene content was found absent in cultivar Safeda and present only at the ripe stage in other cultivars except for cultivar Apple colour. Overall, the cultivar with the least amount of total bioactive compounds was the Apple colour (ripe) and with the highest amount of bioactive compounds was the Safeda (unripe stage), which shows cultivar and ripening both affect the quality of fruits. Therefore, present results showed that guava constitutes higher antioxidant activity and extraction of these bioactive components can be

utilized as a functional food.

Conflict of interest

The authors declare no conflict of interest.

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