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Biochemical changes in guava (*Psidium guajava*) cv. Allahabad Safeda fruits as a function of maturity stages

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ABSTRACT

The effects of maturity stage and harvesting season were investigated on fruit quality attributes like total antioxidant activity (DPPH), total flavanoids, total phenolics, H_2O_2 scavenging activity and ascorbic acid content in fruits of guava (*Psidium guajava* L) cv. Allahabad Safeda obtained from ICAR-CSSRI, Karnal guava orchard. Based on fruit colour and firmness, fruits representing four distinct maturity stages, i.e., immature, mature, ripe and overripe; and two crop seasons, i.e. summer and winter; were selected for biochemical analysis. Significant differences were observed in the antioxidant activities and chemical composition of the fruits at different maturity stages during summer and winter seasons of harvesting. Guava fruits collected in winter season showed higher antioxidant activity, flavanoids content, total phenolics, H_2O_2 scavenging activity and ascorbic acid content as compared with summer season fruits. Immature fruits had higher antioxidant activity, flavanoids content and total phenol content (49.35%, 26.98 mg/100g and 24.84 mg TA/g) during both the seasons than mature (40.02%, 24.22 mg/100g and 23.95 mg TA/g), ripe (31.76%, 20.54 mg/100g and 23.62 mg TA/g) and overripe fruits (25.7%, 17.04 mg/100g and 23.17 mg TA/g), respectively. In contrast, H_2O_2 scavenging activity increased with maturity in both the seasons and was found to be maximum in over ripe fruits (501.48 µmol/g). Ripe fruits had the highest ascorbic acid content (205.03 mg/100 g) that considerably decreased in over ripe fruits (137.25 mg/100 g). These findings indicated that both maturity stage and harvesting season significantly influenced fruit biochemical composition in guava cv. Allahabad Safeda. Season and maturity stage-specific variations in fruit quality assume significance from post-harvest management and consumer preference perspectives.

Key words: Ascorbic acid, Hydrogen peroxide, *Psidium guajava*, Total antioxidant activity, Total phenols

Fruits constitute an integral part of the human diet for daily requirements of health protective compounds such as vitamins, minerals and antioxidants. Given their pivotal role in human health and nutrition, it is desirable to understand and improve the organoleptic quality of fruits which depends on factors such as palatability, taste and aroma as well as the levels of beneficial biomolecules comprising of a range of vitamins, minerals and natural antioxidants like carotenoids, flavonoids, isoflavones, anthocyanins, coumarins, tannins, lignins and catechins (Ramasamy and Thomas 2010). Guava (*Psidium guajava* L.; Myrtaceae) (Ali *et al*. 2004), is one of the most important fruit crops of India. In 2014-15, guava production was 3.99 million tonnes from 2.46 lakh ha area (Anonymous 2015). Guava fruits are rich in valuable antioxidant compounds such as polyphenols (Thaipong *et al*. 2006), ascorbic acid (Kwee and Chong 1990), and carotenoids (Mercadante *et al*. 1999). High levels of ascorbic acid (50–300 mg/100 g fresh weight), which is three to six times higher than that of oranges, make guava fruit a cheap source of this health protective compound. As with other crops, factors such as genotype, fruit growth stage and agro-climatic conditions affect the chemical composition of guava fruits (Padula and Rodriguez-Amaya 1986, Razzaque *et al*. 2000). Many of the natural antioxidants, especially flavonoids, exhibit antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic, and vasodilatory actions (Cook and Sammon 1996). Guava fruits are rich in dietary fibres and vitamin C and have moderate levels of folic acid. Multiple health benefits of guava fruits in terms of improving the immune system and protection against common infections and pathogens has been reported. The anti-carcinogenic property of guava might be due to the presence of compounds like lycopene, quercitin, vitamin C and various polyphenols. In addition, guava also shows anti-inflammatory action and ability to inhibit inflammatory molecules. The antioxidant activity of guava fruits is mainly linked to both total phenolics and ascorbic acid contents (Ravi and Divyashree 2014). In spite of well documented benefits of guava fruit *vis-à-vis* the human health, least is known about the diverse factors

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that influence the quality of the harvested fruit. As factors such as crop season, variety, maturity stage and climatic conditions influence the photochemical compositions of fruits (Cordenunsi *et al*. 2002) that greatly determine fruit quality, shelf-life and consumer acceptability, it is important to investigate the influence of such factors on fruit quality in guava. In light of these facts, we investigated the effects of different maturity stages on antioxidant levels, contents of total and specific phenolic compounds, and ascorbic acid in guava fruits. This information may be useful for guava growers, consumers and processing industries to select the optimum maturity stage and harvesting season for fresh consumption as well as for the purpose of value addition in terms of better fruit quality for high profitability.

MATERIALS AND METHODS

The guava fruits of different maturity levels were harvested from twelve year old guava orchard grown raised in partially reclaimed alkali soils at ICAR-Central Soil Salinity Research Institute, Karnal (latitude 29.43, longitude 76.58, altitude 245 m elevation above the mean sea level). Guava fruits of cultivar Allahabad Safeda were collected in two different seasons, i.e. summer (July-August) and winter (November-December) 2015. The fruits sampled (free of any visible defects) were collected from each direction (N-S and E-W) as well as from the centre of trees. Collected fruits were washed thoroughly in sterilized distilled water and residual moisture was evaporated at the room temperature. The fruits were categorized on the basis of visual observation of colour, size and firmness as shown in Table 1.

Aqueous extracts of four different stages, i.e. immature, mature, ripe and over ripe guava fruits were obtained by homogenizing 2.5 g of guava tissue (pulp and peel) in 25 ml of 80% ethanol until uniform consistency was achieved, using a homogenizer. The homogenates were filtered using Whatman No. 42 filter paper and then stored at -20°C until analysis.

The free-radical scavenging activity of fruit extracts was measured as decrease in the absorbance of methanol solution of DPPH (Andrews *et al*. 2000). A stock solution of DPPH (33 mg/l) was prepared in methanol, which gave initial absorbance of 0.493, and 5 ml of this stock solution was added to 1 ml of extract solution at different concentrations (250, 500, 1 000, 1 500, 2 000 and 2 500 µg/ml). After 30 min, absorbance was measured at 517 nm and compared with standards (10-50 µg/ml). Scavenging activity was expressed as the percentage inhibition calculated using the following formula:

$$\% \text{ Antioxidant activity} = \frac{(\text{Control Absorbance} - \text{Sample Absorbance})}{\text{Control Absorbance}} \times 100$$

where, control absorbance = 0.493

Total flavonoid content was estimated by the method proposed by Delcour and Devarebeke (1985) using the chromogen reagent, with catechin as standard. Absorbance was measured at 640 nm and results were expressed in mg of catechin equivalents (CE) 100/g FW.

Total phenols were estimated by the method of Swain and Hills (1959). Extract (1 ml) was taken in a test tube and diluted with distilled water (7.5 ml). The content was mixed well and 0.5 ml of diluted Folin-Ciocalteau reagent was added to this. Samples were vortexed and after 3 minutes 1 ml of saturated sodium carbonated + 500 µl H_2O was added to make the volume to 10 ml with distilled water. Samples were incubated for one hour and the absorbance was measured at 725 nm taking distilled water as blank. The standard curve was prepared using tannic acid as standard (µg/ml) and data was expressed as mg/g dry weight.

The amount of hydrogen peroxide (H_2O_2) was estimated by the method of Sinha (1971). Fruit tissue (3 g) was macerated in 5 ml of ice cold 0.01 M phosphate buffer (pH 7.0). The homogenate was centrifuged at 10 000 g for 20 min; supernatant was collected and used for estimating H_2O_2 content. Sample (50 µl) was added to 1.95 ml of 0.01 M potassium phosphate buffer (pH 7.0). Two ml of 5% potassium dichromate and glacial acetic acid (1:3 v/v) was added to the mixture. The OD was read at 570 nm against the reagent blank without sample extract. The quantity of H_2O_2 was determined from the standard curve prepared by taking different concentrations of hydrogen peroxide ranging from 20 to 100 µmoles.

Ascorbic acid content was estimated according to the method of Roe (1964) which is based on the reduction of 2, 6-dichlorophenol indophenol dye (2, 6-DCPIP) by standard ascorbic acid solution. Tissue (1 g) was macerated with 20 ml of 3% meta-phosphoric acid in a pestle and mortar. The homogenate was filtered through filter paper and used for estimation. Five ml of the extract was titrated with 0.025% 2, 6-DCPIP dye until a pink color appeared at the end point. The quantity of ascorbic acid was calculated by comparing amount of 2, 6-DCPIP reagent used for unknown sample with that used for known quantities of standard 0.1% ascorbic acid.

All the data were subjected to variance analysis using the SAS (Version 9.3, SAS Institute Inc., Cary, NC, USA). Least significant difference test was applied at 5% probability level to compare the mean differences using randomised block design.

Table 1 Changes in fruit skin color and firmness in the course of ripening

Maturity stage	Degree of maturation	Color	Firmness
1	Immature Green	Dark green	Very hard
2	Mature Green	Greenish	Hard
3	Ripe (Colour Turning)	Pale yellow	Soft
4	Overripe	Translucent grayish	Soft

RESULTS AND DISCUSSION

Total antioxidant activity

Antioxidant activity of guava fruits was measured in

Table 2 Total antioxidant activity during different harvesting seasons and stages of maturity in guava fruits

Season	Total antioxidant activity (%)				
	Immature	Mature	Ripe	Overripe	Means
Summer	47.26	36.43	26.37	22.31	33.09[B]
Winter	51.44	43.61	37.16	29.09	40.32[A]
Mean	49.35[A]	40.02[B]	31.76[C]	25.7[D]	
CV	Level of maturity (T) – 2.645		Season of harvesting (V) – 3.112		
LSD	V–1.05	T–0.85	V×T–1.54	T×V – 1.49	

Least significant difference test was applied at 5% probability level to compare the mean differences.

Table 3 Total flavonoids content during different harvesting seasons and stages of maturity in guava fruits

Season	Total flavonoids content (mg/g FW)				
	Immature	Mature	Ripe	Overripe	Mean
Summer	25.97	24.09	19.31	16.14	21.38
Winter	28.02	24.35	21.78	17.94	23.02
Mean	26.99[A]	24.22[AB]	20.54[BC]	17.04[C]	
CV	Level of maturity (T) – 18.843		Season of harvesting (V) – 21.756		
LSD	V – 4.46	T – NS	V × T – NS	T × V – NS	

Least significant difference test was applied at 5% probability level to compare the mean differences.

terms of its radical scavenging potential. DPPH is a stable free radical and the assay can accommodate a large number of samples in a short period of time. Changes in total antioxidant activity during ripening of guava fruit are shown in Table 2.

It was observed that antioxidant activity in guava fruits decreased from immature green to over ripe stages of maturity in both the seasons of harvesting. DPPH radical scavenging activity of fruits varied among the maturity stages and harvesting seasons. The highest antioxidant activity of guava fruits was found in the immature fruits (47.26, 51.44%) which decreased significantly at mature (36.43, 43.61%), ripe (26.37%, 37.16%) and overripe (22.31, 29.09%) stages in summer and winter season of harvesting, respectively. Similar results with respect to decrease in the antioxidant activity with maturity and ripening have also been reported in pomegranate (Kulkarni *et al*. 2004), peach (Zheng *et al*. 2007b), mango (Zheng *et al*. 2007a) and longan (Duan *et al*. 2007).

Total flavonoids content

The total flavonoid content was significantly higher (26.99 mg/100 g) in immature fruits followed by the mature (24.22 mg/100 g), ripe (20.54 mg/100 g) and over ripe (17.04/mg 100 g) fruits, respectively as shown in Table 3. However, winter season harvesting showed higher total flavonoids (23.02 mg/100 g) content as compared with the fruits harvested in the summer season (21.38 mg/100 g). These results are in conformity with the findings of Ben-ahmed *et al*. (2009) who reported that unripe olive fruits had higher concentrations of flavoinoids as compared to those harvested fully ripe which may be due to formation of complex phenolic compounds such as tannins and lignin in the later stages of ripening. Jaffery *et al*. (2003) found that variation in total flavonoids contents of guava at different stages of maturity might be due to the presence of phenolics which is influenced by climatic conditions, species, soil characteristics and the stage of harvesting.

Total phenolics

The changes in total phenolic content during different maturity stages of guava fruit in summer and winter seasons of harvesting are presented in Table 4. Phenol concentration was significantly higher at the immature stage (24.48 mg TA/g), while minimum concentration (23.17 mg TA/g) was found in over ripe fruits.

In contrast, concentration of total phenolics showed non-significant differences among different seasons of harvesting. Still, the phenolics content was marginally higher in winter season fruits compared to summer season fruits regardless of the stage of maturity. These results are in accordance with the findings of Parr and Bowell (2000) who reported that decrease in total phenols in over-ripe fruits compared to the ripe ones might be caused by an amplified polyphenol oxidase activity. Kalt *et al.* (2003) and Castrejon *et al*. (2008) also reported decrease in the phenolic contents during ripening in high bush blueberries. Total phenol content decreased gradually from immature to overripe stages of maturity in medlar fruits (Rop *et al*. 2011). Stanislaw (1968) found that loss in astringency during ripening and decrease in total phenol content seemed to be due to increased polymerization of leucoanthocyanidins and hydrolysis of the astergingent arabinose ester of hexahydrodiphenic acid.

Hydrogen peroxide content

Data presented in Table 5 show the H_2O_2 content of guava fruits at various stages of maturity during two different harvesting seasons. H_2O_2 content increased with the advancement of maturity stage during summer and winter seasons, respectively. At immature green stage, H_2O_2 content was the lowest and increased with the maturity during both

Table 4 Total phenolic content during different harvesting seasons and stages of maturity of guava fruit

Season	Total phenolic content (mg TA/g)				
	Immature	Mature	Ripe	Overripe	Means
Summer	24.17	23.80	23.37	22.95	23.57
Winter	24.79	24.11	23.86	23.39	24.04
Mean	24.48[A]	23.95[B]	23.62[B]	23.17[C]	
CV	Level of maturity (T) – 2.636		Season of harvesting (V) – 1.703		
LSD	V – 0.37	T – NS	V × T – NS	T × V - NS	

Least significant difference test was applied at 5% probability level to compare the mean differences.

Table 5 H_2O_2 content during different harvesting seasons and stages of maturity of guava fruits

Season	H_2O_2 (µmol/g FW)				
	Immature	Mature	Ripe	Overripe	Means
Summer	306.24	396.17	448.54	492.37	410.83
Winter	336.98	418.94	459.36	510.59	431.47
Mean	321.61^{D}	407.56^{C}	453.95^{B}	501.48^{A}	
CV	Level of maturity (T) – 6.431		Season of harvesting (V) – 3.188		
LSD	V – 12.39	T – NS	V × T - NS	T × V - NS	

Least significant difference test was applied at 5% probability level to compare the mean differences.

Table 6 Ascorbic acid content during different harvesting seasons and stages of maturity of guava fruit

Season	Ascorbic Acid (mg/100 g)				
	Immature	Mature	Ripe	Overripe	Means
Summer	118.39	145.79	198.44	122.85	146.37^{B}
Winter	133.41	158.36	211.63	151.65	163.76^{A}
Mean	125.9^{D}	152.08^{B}	205.03^{A}	137.25^{C}	
CV	Level of maturity (T) – 9.239		Season of harvesting (V) – 5.624		
LSD	V – 8.05	T – 12.58	V × T - NS	T × V - NS	

Least significant difference test was applied at 5% probability level to compare the mean differences.

the seasons of harvesting. H_2O_2 content significant increased from 306.24 µmol/g at immature green stage to 492.37 µmol/g in overripe fruits during summer season and 336.98 µmol/g at immature green stage to 510.59 µmol/g at overripe maturity stage, respectively. H_2O_2 content was found more during winter season as compared to summer season of harvesting.

Hydrogen peroxide is one of the toxic reactive oxygen species, which is also generated during normal metabolic processes. Catalase (H_2O_2 degrading enzyme) is one of the primary enzymatic defenses against oxidative stress induced by senescence (Zimmermann *et al*. 2006). However, catalase activity increased continuously during ripening of tomato fruits (Andrews *et al*. 2004, Mondal *et al*. 2004) whereas in saskatoon (Rogiers *et al*. 1998) and orange (Huang *et al*. 2007), it was found to decrease continuously. A gradual increase in hydrogen peroxide content was also observed in longan (Duan *et al*. 2007) and mango (Zheng *et al.* 2007a). However, Mondal *et al*. (2004) reported that H_2O_2 content only marginally decreased during ripening in tomato. These findings tend to show that H_2O_2 content either increases or decreases or even remains stable during fruit ripening in different species.

Ascorbic acid

Results presented in Table 6 revealed that ripe fruits had the maximum ascorbic acid content (198.44 and 211.63 mg/100 g FW during summer and winter seasons, respectively). However, its minimum contents (118.39 and 133.41 mg/100 g FW during summer and winter season, respectively) were recorded in the immature fruits.

These results are supported by the observations of Mondal *et al.* (2004) who noted increase in fruit ascorbic acid content in tomato up to turning stage followed by a decline at the ripe stage. Ascorbic acid content was reported to increase during the ripening of jujube (Bal and Josan 1980) and banana (Mustaffa *et al*. 1998) while a continuous decline has been observed in sapota (Lakshminarayan and Subramanyam 1966) and cherry (El-Bulk *et al*. 1997, Kadioglu and Yavree 1998). In guava fruits also (Dhillon *et al*. 1987), ascorbic acid content has been reported to show a sigmoidal increase during ripening. These findings are also in agreement with those of Zheng *et al.* (2007b) in peach and Zheng *et al.* (2007a) in mango. El- Buluk *et al.* (1997) reported that ascorbic acid content increased slowly during initial growing period and significantly decreased during maturation and ripening in different guava cultivars. Ascorbic acid in fruits of guava cvs. L-49 and Banarsi Surkha increased up to colour turning stage and then decreased dramatically at over-ripe stage (Jain *et al*. 2003). Ascorbic acid content of ber fruits increased during ripening on tree as well in storage (Kannan and Thirumaran 2003). Nunes *et al.* (2006) compared the ascorbic acid content during ripening of strawberries in storage as well as in field and reported that the total ascorbic acid content of fruits increased during ripening and development irrespective of the ripening conditions. Thakur *et al.* (2002) observed higher retention of ascorbic acid in Kinnow fruits stored at low temperature as compared to fruits stored at ambient temperature.

The investigation was undertaken to ascertain the correlation between harvesting season and different stages of maturity on the antioxidant activity, phenolic compounds and ascorbic acid content of guava cv. Allahabad Safeda. Fruits were collected at four different stages of ripening, i.e. immature green (IG), mature green (MG), ripe (R) and over ripe (OR). We found that maturity stages and harvesting seasons differently modulated the biological activity and the chemical composition of guava fruits. For example, winter season guava fruits showed higher total antioxidant activity, total flavonoids content, H_2O_2 content and ascorbic acid content which is related to the development cycle during various stages of maturity. Our findings assume significance from the processors perspective in that this information may be helpful in selecting the quality fruits for value addition. Although, organoleptic traits are the major determinants of fruit quality from consumer's perspective, it is possible that consumption of winter season guava fruits will be more beneficial than those from summer season crop.

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