Indian Journal of Agricultural Sciences **89** (11): 1797–1801, November 2019/Article
https://doi.org/10.56093/ijas.v89i11.95292

Bioactive compositions in guava (*Psidium guajava*) at different stages of maturation in arid conditions

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Received: 24 March 2018; Accepted: 23 July 2019

ABSTRACT

The bioactive compounds, total antioxidant activity and minerals in white, pink and red flesh guava (*Psidium guajava* L.) varieties were assessed and quantified at three different stages of maturity in arid conditions. Changes in pectin content followed similar trend as fruit firmness and it gradually decreased till ripe stage. Total soluble solids and total sugars increased till full ripe stage but reverse was case for acidity. Ascorbic acid in white flesh guava varieties increased till colour turning stage and then after it decreased, while in coloured varieties it showed decreasing trend till ripe stage. White flesh varieties contains negligible quantity of anthocyanin, lycopene and total carotenoid while pink flesh (Lalit) had highest concentration of these compound followed by red flesh (Red flesh). Total phenols and total antioxidant activities of guava fruits were comparatively low during green mature stage and it increased progressively in later stages. Flesh colour did not show any definite pattern on phenolic content and total antioxidant activities. Phenols was recorded highest in Allahabad Safeda, while total antioxidant activities in Lalit. Stage of maturation showed variable changes in mineral contents in guava varieties. It can be inferred from the present study that the remarkable metabolic changes occur between mature green to colour turning stage.

Key words: Antioxidants, Arid Conditions, Guava, Maturity Stages, Minerals, Phytochemicals

The guava (*Psidium guajava* L.) of Myrtaceae family is designated as "poorman's apple" owing to its high nutritive values. It is consumed fresh as well as processed. The guava fruits contain sugars, iron, calcium, phosphorus and vitamins in higher concentration. It is rich in dietary fiber, ascorbic acid, phenols, pectin, and carotenoids, particularly lycopene, which are important in the prevention of several human diseases. It is such a hardy fruit tree which is now widely distributed throughout the tropical and subtropical parts of India including western Rajasthan. Guava is cultivated over 2457 ha with a total production of 23.75 MT and a productivity of 93.92 q/ha in the state of Rajasthan (Anonymous 2015). Based on previous varietal evaluation study at ICAR-Central Arid Zone Research Institute Jodhpur revealed that guava can be successfully grown in arid western part of India, and being a remunerative crops farmers are also looking for diversification to enhance their income.

Guava passes through different stages of harvesting as per the consumer preference vis-a-vis differential level of nutritional composition at different stage of maturation (Moreno *et al.* 2014). It is quite obvious that quality and nutritional value of fruits depend on various physiological and biological changes, which occurs during growth and development (Heverton *et al.* 2015). The performance of guava in particular climate with respect to level of bioactive phytochemicals and nutritional composition may vary from cultivars to cultivars and stage of maturation. Knowledge about its phytochemical profiling, antioxidants activities and minerals may be major indicator for deciding food value of guava at particular stage because it is used as table as well as processing products. Consumer's preference about guava is also variable from green to full ripe stage therefore, stage wise chemical analysis of these nutrients is important from health point of view too. Keeping these in background, four varieties having pink, red and white flesh was assessed for their bioactive compounds, antioxidant activity and minerals at three different stages of maturation.

MATERIALS AND METHODS

The study was carried out during 2015–16 at ICAR-Central Arid Zone Research Institute, Jodhpur. Air layered plants of Allahabad Safeda and MPUAT Sel-1 (white flesh), Lalit (pink) and Red Flesh (red flesh) planted at spacing of 5 m × 5 m were considered as experimental material. Three uniform trees of each cultivar were selected and sufficient flowers were tagged randomly in all aspect of tree during *Mrig bahar* crop season. Composite sample of ten fruits were assessed for bioactive phytochemicals (sugars,

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acidity, phenols, anthocyanin, total carotenoid, lycopene, ascorbic acid, pectin), total antioxidant activity, mineral composition (dry matters, calcium, phosphorus, potassium and iron) and firmness.

Fruit firmness was determined by using digital fruit hardness tester. The fruits were punctured at two places opposite to each other in radial axis with a tip of 6 mm and the pressure required was expressed in kg/cm^2. The edible portion of guava fruits weighed together and then oven-dried at 65°C until constant weight. The dry matter content was calculated as a percentage of fresh weight (% FW). Guava fruit juice was collected from pulp to measure total soluble solids by digital hand held refractometer and brix value was noted in per cent at room temperature. The titratable acidity was determined as suggested in A.O.A.C. (1990). The sugar contents was determined by volumetric method using Fehling solution based on the principle that sucrose content of fruit is quantitatively hydrolyzed to glucose and fructose in the presence of HCL as per the method A.O.A.C. (1990). Ascorbic acid content was determined by using 2, 6-Dichlorophenol indophenol dye method (A.O.A.C.1990). Total anthocyanins were extracted from guava fruit using the method described by Ranganna (2001). Total anthocyanin concentration was expressed as mg/100 g fresh weight. The total pectin, Lycopene and carotene content were estimated as per the method given by the Ranganna (2001).

The amount of total phenolic in extracts was determined with the *Folin-Ciocalteu* reagent. 0.1–0.2 ml of methanolic extract were taken in test tubes and made up the volume to 4 ml with distilled water. After that, add 0.5 ml of 1N *Folin- Ciocalteu* reagent and mixed well and after that add 2 ml of 6% sodium carbonate solution and mixed with vortex. Incubate the tubes under boiling water bath for 2 min and cooled the tube at room temperature, the absorbance against prepared reagent blank was determined at 650 nm with an UV-Vis spectrophotometer. The total phenolic content of sample extracts was expressed as mg catechol equivalents (CtE)/100 g DW. All samples were analyzed in triplicates.

Methanolic extract: Accurately weighed 250 mg of 100 micron mess fine sample powder and homogenized with 10 ml of 90% methanol using pestle and mortar and the homogenate was subjected to centrifuge at 8000 rpm for 10 min at 25°C and collected the supernatant. Re-extract the residue twice with 5 ml of 50% methanol and centrifuge. Pooled the supernatant and made up the volume to 20 ml with 90% methanol and stored at -20°C.

Total antioxidant activity (TAA) in extract was determined by two *in-vitro* systems I; Cupric ion reducing capacity (CUPRAC) assay and ii; DPPH radical scavenging activity. TAA by CUPRAC system was measured in accordance to the method of Apak *et al.* (2004). Simultaneously, added 1 ml each of 10 mM cupric chloride, 75 mM neocuproin and 1 M ammonium acetate (*p*H 7.0) to test tubes containing 2 ml of distilled water. 100 µl methanolic extract was added in each test tube separately. These mixtures were incubated for 30 min at room temperature and measured the OD at 450 nm against the reagent blank. Ascorbic acid was used as positive reference standard and expressed the results as mg AAE/100g. TAA by DPPH radical scavenging activity was assayed by following the method of Re *et al.* (1999). Concentration of major mineral nutrients in guava fruits like calcium (Ca), phosphorus (P), potassium (K) and iron (Fe) were determined in diacid digest with flam photometer and an atomic absorption spectrophotometer using the procedure of Bhargava and Raghupati (1984).

The average value of 3 replications was used for statistical analysis. Differences between samples were tested by analysis of variance (ANOVA) to assess differences group means. P values ≤0.5 were considered significant.

RESULTS AND DISCUSSION

Fruit firmness showed a progressive decline during different stages of maturation in all four varieties (Table 1). The decline in firmness was observed maximum from colour turning stage to full ripe mature stage. However, rate of decline was maximum in Red flesh (5.42–1.89 kg/cm^2) and lowest in Allahabad Safeda (5.93–2.53 kg/cm^2). Among the varieties, the firmness was highest in MPUAT Sel-1 followed by Allahabad Safeda at mature green stage and lowest in Red Flesh at full ripe stage. The dry matter content in guava fruits was gradually increased up to colour turning stage in all the varieties, after which it started decline during full mature stage (Table 1). The maximum dry matter recovery was recorded at colour turning stage followed by green mature and lowest in full mature fruits of all the four guava varieties studied. Changes in pectin content followed similar trend as recorded in fruit firmness which gradually decreased from green mature stage till full mature stage in all the varieties except Lalit in which it increased up to colour turning stage then after it started decreasing but even though it was higher than green mature stage (Table 1). Interestingly pectin content was found highest in Allahabad Safeda at green mature stage followed by Red Flesh but at full ripening it was highest in Lalit and lowest in MPUAT Sel-1. Therefore, results ascertain that rate of degradation of pectin was lowest in Lalit and highest in MPUAT Sel-1. The reduction in fruit firmness from green mature to full ripe stage reflects changes in cell wall degradation due to action of hydrolytic enzyme on cell wall. Paliyatha *et al.* (2008) also stated that causes of softening of several fruits are because of cell wall degradation associated with degradation of cellulose or pectin compound or both. It is well supported by the trends in changes in pectin content observed in present study. Irrespective of cultivars, during ripening there is an increase in the synthesis of polygalacturonase and pectin methyl esterase enzymes that act solubilizing pectin thus determining the decrease in stiffness and firmness (Azzolini *et al.* 2004, Pereira *et al.* 2006).

A linear increase in TSS was noticed till full maturity stage in all guava varieties studied. Total soluble solids were comparatively low at green mature stage but even it was in acceptable range. At full mature stage the highest amount of TSS (16.8%) was observed in MPUAT Sel-1 followed

Table 1 Changes in fruit firmness, dry matter and biochemical content in *P. guajava* fruits at different stages of maturation

Variety/ Stage of maturation	Fruit firmness (kg/cm^2)	Dry matter (%)	TSS (%)	Pectin content (%)	Acidity (%)	Total sugars (%)	Anthocyanin (mg/100g)	Ascorbic acid (mg/100)
Allahabad Safeda								
Green mature	5.93	15.06	9.6	1.50	0.653	8.92	0.327	202.5
Colour turning	4.21	17.08	11.4	1.36	0.614	12.0	0.431	214.6
Ripe	2.53	14.1	14.9	1.26	0.461	13.04	0.472	193.8
Lalit								
Green mature	4.63	16.42	10.0	1.22	0.60	6.63	0.69	188.8
Colour turning	3.36	17.0	12.5	1.30	0.55	10.6	0.70	162.5
Ripe	2.60	14.8	13.2	1.27	0.474	11.7	0.88	167.8
Red Flesh								
Green mature	5.42	15.91	8.3	1.30	0.512	5.27	0.59	178
Colour turning	2.6	16.22	10.2	1.26	0.486	7.8	0.672	163.5
Ripe	1.89	13.1	12.8	1.12	0.371	9.8	0.82	146.9
MPUAT Sel-1								
Green mature	6.98	15.43	10.2	1.16	0.678	7.56	0.352	180
Colour turning	4.5	17.79	13.0	1.08	0.55	10.28	0.514	196.5
Ripe	3.1	16.16	16.8	1.02	0.538	14.1	0.632	149.6
CD (P=0.05)	0.68	0.87	1.9	0.14	0.18	1.2	0.12	8.43

by Allahabad Safeda and lowest in Red Flesh (12.8%). Likewise TSS, considerable changes in sugar content was also noticed during different stages of maturation. There was increasing trend in sugar content till full maturity but it was remarkably increased from green mature to colour turning stage in all four varieties. Results showed that white flesh varieties i.e. Allahabad Safeda and MPUAT Sel-1 accumulated reasonable quantity of sugars even at green mature stage which was significantly higher than coloured varieties. In contrast to TSS and total sugars, a gradual decrease in titratable acidity was recorded from green mature till full ripe stage. Rate of decrease from green mature to colour turning stage was meager in all varieties but it significantly decreased during later stage till full maturity. A varietal variation was also noticed as acidity ranged from 0.678–0.538% in MPUAT SEl-1 and lowest in Red Flesh (0.512–0.371%). Irrespective of maturity stages, acidity was found higher in white flesh variety followed by pink and lowest in Red flesh. Results are in accordance with the findings of Bashir *et al.* (2003) and Singh and Jain (2007). Higher TSS and total sugars in white flesh guava than coloured flesh varieties might be due to genetic characters as Patel *et al.* (2013) also reported similar findings in white and pink flesh guava varieties. Present findings on changes of titratable acidity was in order with Soaren *et al.* (2007), who also reported that in general, young fruits of guava contain more acids that declined throughout maturation until ripening. Dry matter accumulation with respect to maturation stage may be another factor responsible for variable sugar and acid in guava. It is well supported by present findings also as dry matter accumulation was more in white flesh varieties than coloured varieties and it followed increasing trend up to colour turning stage.

Data shown in Table 1 indicates a rapid increase in the anthocyanin concentration in guava varieties between green mature to colour turning stage then after a steady increase till full ripe stage in coloured varieties. However, the highest anthocyanin concentration was recorded at full mature stage in all the varieties which followed order of pink flesh>red flesh>white flesh. Varietal trend showed highest concentration in Lalit followed by Red Flesh and lowest in Allahabad Safeda. It was obvious to note that colored varieties had higher concentration of anthocyanin even at green mature stage than white flesh varieties at full mature stage. Ascorbic acid content in white flesh guava varieties increased till colour turning stage and then after decreased during fruit ripening. While in pink and red flesh varieties it showed steadily decreasing trend from green mature stage to till full mature stage. The content of ascorbic acid in white flesh (Allahabad Safeda and MPUAT Sel-1) reached a maximum level at colour turning stage while, it was maximum at green stage itself in coloured flesh varieties (Lalit and Red Flesh). Among all the tested varieties, maximum amount of ascorbic acid was recorded in Allahabad Safeda and lowest in Red Flesh, irrespective of stage of maturation (Table 1).

Guava varieties showed increasing trend of total carotenoid during maturation and it was recorded highest at full mature stage in all four varieties studied (Fig 1). However, pink and red flesh varieties had significantly much higher level of carotenoid than white flesh varieties. Likewise, total carotenoid, lycopene content in guava varieties followed similar trend. However, negligible amount of lycopene was detected in white flesh varieties at green as well as colour turning stage. Among the coloured varieties, pink flesh i.e. Lalit recorded comparatively higher concentration of lycopene than red flesh during different stages of maturation. Similar to anthocyanin, coloured varieties had higher concentration of carotenoid and lycopene even at green mature stage than white flesh varieties at full mature stage. Fig 1 shows a rapid increase in phenolic content from green mature to colour turning

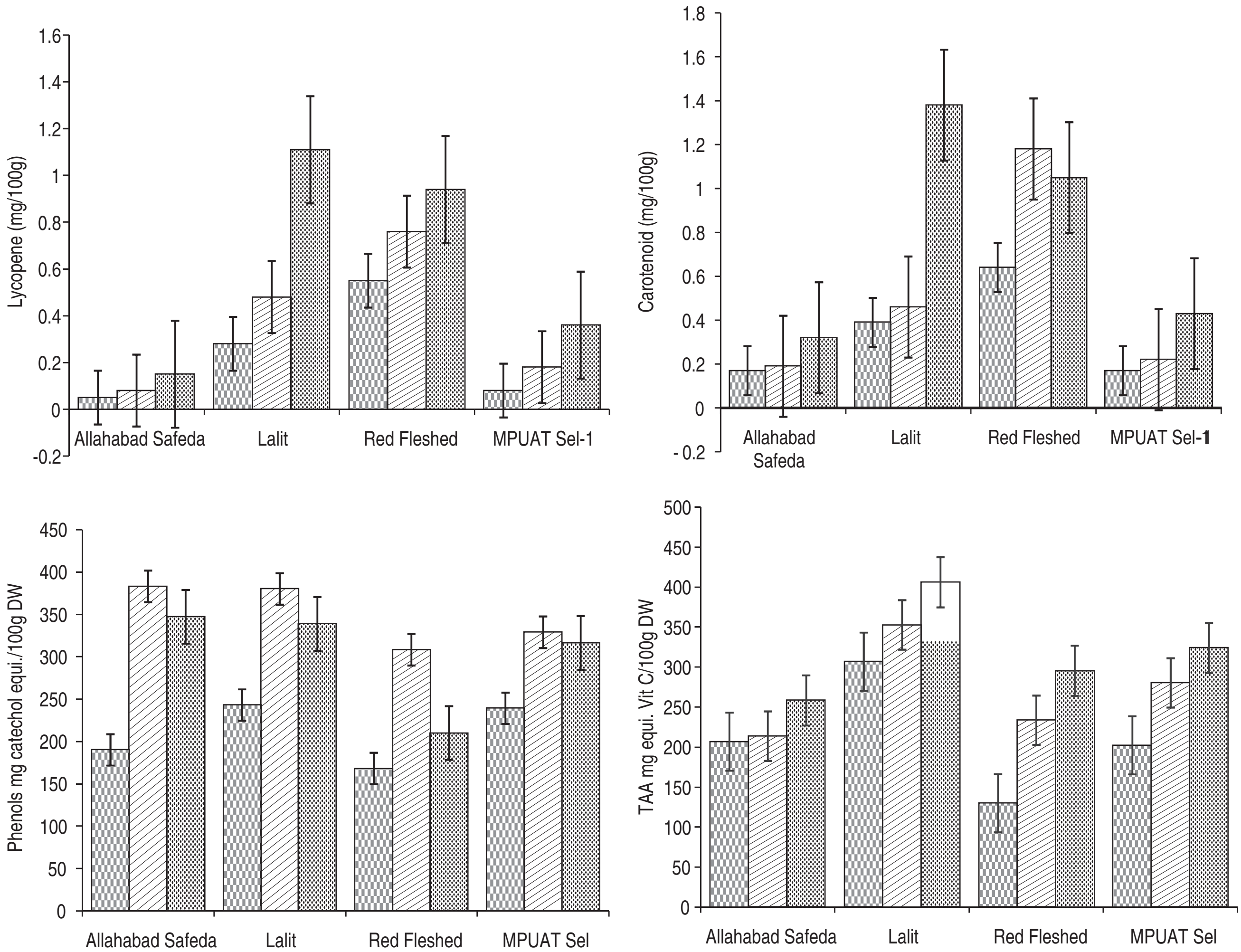

Fig 1 Lycopene, carotenoids, phenols and total antioxidant activity in *P. guajava* fruits at different stages of maturation. Vertical bar represents ± standard error

stage and then after gradual decrease till full mature stage in all four guava varieties. Flesh colour did not show any definite pattern on phenolic content. The highest level was recorded in Allahabad Safeda followed by Lalit at colour turning stage while at green mature stage it was highest in Lalit followed by MPUAT Sel-1. Total antioxidant activities of guava fruits were comparatively low during green mature stage and it increased progressively during latter stages of maturation till ripening in all four varieties. Similar to phenolic content, flesh colour of varieties did not show any definite pattern on total antioxidant activity. It was observed highest in pink flesh (Lalit) followed by red skin (MPUAT Sel-1) while lowest in Allahabad Safeda (white flesh) at full mature stage. Unlike other varieties, Allahabad Safeda did not show much variation for total antioxidant activity across the maturity stages. The active synthesis of ascorbic acid during fruit development and early ripening in present study supported the fact that it might be attributed to inactivation of ascorbic acid oxidase due to high phenol content during early maturation stage. Similar to present result, Bashir *et al.* (2003) also reported higher values of ascorbic acid in white than coloured guava. As anthocyanin is one of the pigment responsible for colour in guava fruits and it has definite relation with intensity of colour (Heverton *et al.* 2015). Content of anthocyanin increased towards maturity with the record of highest value in pink followed by red coloured varieties. Therefore, the ripening stage may be a major factor for anthocyanin levels in guava together with the cultivars as observed in present study also. Another pigment responsible for colour in guava peel and pulp is lycopene and biosynthesis of lycopene increased with maturation (Azzolini *et al.* 2004). Heverton *et al.* (2015) reported that the colour changes of guava pulp are associated with enzyme activity with lycopene production which increases during ripening. This might be the reason coloured varieties had more lycopene as well as anthocyanin content and it progressively increased with ripening. Rapid increase in phenol content during green stage might be due to increase of its biosynthesis initially but decrease in later stage due to increase in activity of polyphenol oxidase. The result on total phenolics was in agreement with the findings of Yan *et al.* (2006) and Reddy *et al.* (2010) in guava. Carotenoids including lycopene, anthocyanin, ascorbic acid, phenolics, tannins either alone or in combination are responsible for

Table 2 Changes in mineral content in *P. guajava* fruits at different stages of maturation

Variety/Stage of maturation	P	K	Ca	Mg	Fe
Allahabad Safeda					
Green mature	10.31	322.4	35.07	18.83	4.69
Colour turning	13.22	339.2	30.76	19.67	4.84
Ripe	14.29	343.7	23.04	18.59	5.83
Lalit					
Green mature	10.37	326.1	30.33	17.30	4.93
Colour turning	11.29	353.9	26.39	19.05	4.83
Ripe	13.29	359.9	19.89	18.72	5.26
Red Flesh					
Green mature	9.97	326.8	28.69	20.07	5.03
Colour turning	12.01	339.2	22.40	20.94	5.09
Ripe	13.24	348.8	20.36	22.26	5.86
MPUAT Sel-1					
Green mature	11.37	318.1	36.77	19.04	4.94
Colour turning	12.41	322.8	34.12	19.89	5.00
Ripe	15.32	33.63	28.8	18.20	6.02
CD (P=0.05)	1.87	6.24	2.39	0.18	0.12

the antioxidant activity of guava. The surge in antioxidant activity during colour turning stage onwards might be attributed to an increase concentration of anthocyanin, total carotenoids and lycopene besides reasonable quantity of ascorbic acid and other phytochemicals observed during study. Comparatively higher antioxidant activity during initial stage of maturation in white flesh varieties might be due to more contribution of ascorbic acid and phenolics towards total antioxidant activity. This might be the reason that flesh colour of varieties did not show any definite pattern on total antioxidant activity in guava.

Stage of maturation showed variable changes in mineral contents in guava varieties (Table 2). Phosphorus, potash and iron content in all the varieties showed increasing trend towards ripening whereas, calcium content was decreased from green mature till full mature stage. However, magnesium content in all the varieties increased till colour maturation stage and then after it decreased. Irrespective of maturity stages, phosphorus, calcium and iron content was found higher in white flesh varieties while calcium concentration was higher in coloured varieties. No definite pattern was found for magnesium with respect to flesh colour of guava varieties. Highest concentration of phosphorous and iron at full mature stage and calcium at green mature stage was recorded in MPUAT Sel-1 followed by Allahabad Safeda. However, potassium and magnesium was higher at full mature stage in Lalit and Red flesh, respectively.

It is concluded from the present study that the remarkable metabolic changes occurred between mature green to colour turning stage except lycopene and anthocyanin, implying that for maximum food value and improved postharvest handling (guava fruits may be harvested at colour turning stage). However, colourded varieties for processing purposes may be harvested at full ripe stage for maximum lycopene and anthocyanin content.

REFERENCES

Anonymous. 2015. Directorate of Horticulture, Govt. Rajasthan.

AOAC. 1980. *Official Methods of Analysis*. Association of official analytical chemists. Washington, D.C. USA.

Apak R, Guclu K, Ozyurek M and Karademir S E. 2004. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry* **52**: 7970–81.

Azzolini M, Jacomino A P and Spoto M H F. 2004. Maturation stages and quality postharvest of guavas 'Pedro Sato'. *Revista Brasileira de Fruticultura* **26**: 29–31.

Bashir Hind A, Abu-Goukh and Abu-Bakr A. 2003. Compositional changes during guava fruit ripening. *Food Chemistry* **80**: 557– 63

Bhargava B S and Raghupati H B. 1984. Analysis of plant materials for macro and micronutrients, pp 49–82. (Eds) Tandon H.L.S. *Methods of Analysis of Soils, Plants, Water and Fertilizers*. Fertilizers development and consultation organization, New Delhi.

Heverton M A, Fabiola F G R, Wegila D C, Carla de F A N, Aline A B, Margareth L A and Jose G M C. 2015. Chemical profile and antioxidant capacity verification of *Psidium guajava* (myrtaceae) fruits at different stages of maturation. *Excli Journal* **14**: 1020–30

Moreno M A, Zampini C, Costamanga M, Sayago J E, Ordonez R M and Isla M I. 2014. Phytochemica composiyion and antioxidant capacity of *Psidium guajava* fresh fruits and flour. *Food and Nutrition Sciences* **5**: 725–32

Paliyath G and Murr D P. 2008. Postharvest biology and technology of fruits, vegetable and flowers, pp 19–50 . *Biochemistry of Fruits*. (Eds.) P. Gopinadhan, D P Murr, A K Handa, S. Lurie. Wiley-Blackwell publishing, Iowa, USA.

Patel R K, Maiti C S, Deka B C, Deshmukh N A and Nath A. 2013. Changes in sugars, pectin and antioxidants of guava *(Psidium guajava)* fruits during fruit growth and maturity. *Indian Journal of Agricultural Sciences* **83**(10): 1017–21

Pereira T, Carlos L A, Oliveira J G and Monteiro A R. 2006. Influence of storage conditions on the physical and chemical characteristics of guava (*Psidium guajava*), cv. Cortibel with white flesh. *Ceres* **53**: 276–84

Ranganna S. 2001. *Handbook of Analysis and Quality Control for Fruits and Vegetable Products*. 2nd edn. Tata McGraw hill publication company Ltd. New Delhi, India.

Re R, Pelledrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine* **26**: 1231–37

Reddy C, Vijay Kumar, Sreeramulu D and Raghunath M. 2010. Antioxidant activity of fresh and dry fruits consumed in India. *Food Research International* **43**: 285–88

Singh P and Jain V. 2007. Fruit growth attributes of guava (*Psidium guajava* L.) cv. Allahabad Safeda under agro-climatic conditions of Chhattisgarh. *Acta Horticulturae* **735**: 335–38

Soares F D, Pereira T M and Monteiro Alcilene R. 2007. Volatile and non-volatile chemical composition of the white guava fruit (*Psidium guajava* L.) at different stages of maturity. *Food Chemistry* **100**: 15–21

Yan Lim Y, Teng Lim Theng and Jhi Tee Jing. 2006. Antioxidant properties of guava fruits: comparison with some local fruits. *Sunway Academy Journal* **3**: 9–20.