Plant Archives Vol. 18 No. 2, 2018 pp. 2363-2371 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF SEASONALITY AND FRUIT RIPENING STAGES ON BIOACTIVE CONSTITUENTS AND ANTIOXIDANT POTENTIAL OF GUAVA FRUIT CULTIVARS

Surabhi Shukla[1], Devinder Kaur[1]*, Seeraptpreet Kaur[2] and Rajendra Prasad[3]

[1*]Centre of Food Technology, University of Allahabad
[2]Khalsa College, Amritsar, Punjab
[3]Kulbhaskar Ashram Degree College, Allahabad

Abstract

Objective : Tropical fruits claim to have phenolic compounds that have been reported to possess strong antioxidant activity. Several studies are going on worldwide directed towards finding natural antioxidants of plant origin. This study explores the phenolic content and antioxidant potential of five edible guava cultivars (Allahabad Surkha, Lalit, Red fleshed, Chittidar and Allahabad Safeda) collected in three different seasons (winter, summer and rainy) during maturity (unripe, semi-ripe and ripe).

Method: Guava fruits were screened for their bioactive components (total phenolic content, total flavonoid content, Vitamin C) as well as for the antioxidant properties by using *vitro* assays (DPPH, ABTS, FRAP).

Result : The findings suggest that the cultivars, ripening stages and seasons significantly affected the bioactive constituents and antioxidant capacity of the fruit. The bioactive constituents and antioxidant capacity decreases as the ripening proceeds (unripe>semi-ripe>ripe) with the exception of vitamin C which increases with the ripening (unripe<semi-ripe<ripe). The study yields maximum amount of these functional components in the winter season followed by the summer and the rainy season.

Conclusion: This study provides information on the bioactive composition, as well as the antioxidant capacities of guava cultivars which is an important commercial fruit. Results obtained showed that major compositional changes in the fruit are developmentally regulated. In general, there were significant differences in the pattern of accumulation of, ascorbic acid while there are significant declines in the levels of total phenolics and antioxidant capacity during fruit development and maturation.

Key words: Gauva, bioactive compounds, maturity stages, antioxidant activity, cultivars.

Introduction

Consumption of tropical fruit is related to lowered incidences of chronic degenerative diseases due to the presence of bioactive compounds with antioxidant properties (Van't Veer *et al*., 2000). *Psidium guajava* L. is one of the most important crops belonging to the genus Psidium and the Myrtaceae family (Joseph *et al*., 2011). Guava (*Psidium guajava* L.) fruit is valued as a potential source of pectin, ascorbic acid (vitamin C), sugars and minerals. Different parts of guava have been traditionally used in the folk medicine of several civilizations. Natural antioxidants, particularly in fruits and vegetables have gained increasing interest among consumers and the scientific community because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer (Renaud *et al*., 1998). The defensive effects of natural antioxidants in fruits and vegetables are related to three major groups: vitamins, phenolics, and carotenoids. Reactive oxygen species (ROS) refer to free radicals involving the oxygen element. The formation of ROS can be quenched by consuming fruits and vegetables due to selective bioactive compounds (Girard *et al*., 2009). It is generally considered that different parameters such as season, variety, stages of maturity and climatic conditions influence the phytochemical composition of fruits (Temple *et al*., 2000). The skin of the fruit and flesh colour varies

***Author for correspondence :** E-mail : devinderkaur02@gmail.com

between cultivars depending on the type and amount of pigments. The nutritional and health-promoting properties of *Psidium guajava*, 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 polyphenol composition for the intended use of the fruit. Therefore, the present research work aimed at evaluating the antioxidant attributes and bioactive constituents of guava cultivars assayed at different stages of maturity harvested at three seasons.

Materials and methods

Plant materials

Guava cultivars were collected at different ripening stages in various seasons (winter, summer, rainy) from an orchard in Khusroobagh (under the Department of Horticulture and Food Processing, Uttar Pradesh Government), Allahabad and stored at 15±2°C with relative humidity of 90-95%.

Preparations of fruit extracts

Fresh fruits (2.0 g) were extracted with 5 ml of aqueous ethanol (ethanol: water 50:50v/v) for 24 hours at room temperature in orbital shaker (REMI, C1S-24BL). The extracts were separated from the residues by filtering through Whatmann No.1 filter paper. The residues were extracted twice with the fresh solvent and extracts were combined. The extracts were stored in a refrigerator (5±2^{0}C) until used for further analysis.

Total phenolic content

Total phenolic content was determined by the Folin Ciocalteau method using gallic acid monohydrate as standard. It was dissolved in various extraction solvents. An aliquot (0.05 ml) of sample or standard was placed in test tube and the volume was adjusted to 6 ml with deionised water. Then 0.3 ml of Folin Ciocalteau was added to all tubes. After 8 minutes 0.9 ml of 20% sodium carbonate was added to the mixture and then incubated for 30 minutes at 40°C. Absorbance of the resultant blue color was measured at 765 nm in spectrophotometer (Model: Evolution 600, Thermoscientific, US). Total phenolics were expressed as mg gallic acid equivalent/ gm weight.

Ferric reducing power

Ferric reducing power assay was used to determine antioxidant activity. The FRAP assay was done according to Ahmad *et al*. with some modifications. The stock solutions included 300 mM acetate buffer pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3.6H_2O$ solution and then warmed at 37°C before using. Fruit extracts (150mL) were allowed to react with 2850 µL of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were then taken at 593 nm. The standard curve was linear between 25 and 800 mM Gallic acid. Results are expressed in mg GAE/ g. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve.

DPPH radical scavenging activity

Free radical scavenging activity of extracts was measured by the slightly modified method of Alothman *et al*. The antioxidant capacity of the fruit extracts was studied through the evaluation of the free radical scavenging effect on the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. An aliquot (100 µl) of fruit extract was mixed with 3.9 ml of 0.1 mM DPPH methanolic solution. The mixture was thoroughly vortex-mixed and kept in the dark for 30 min. The absorbance was measured at 515 nm. Results were expressed as percentage of inhibition of the DPPH radical. Ascorbic acid was used as standard.

Flavonoid content

Total flavonoid contents were measured with the aluminum chloride colorimetric assay as suggested by Boateng *et al*. Ethanolic extracts (2 ml) were mixed with 150 µl of sodium nitrite (5%). After 5 minutes, 150µl of aluminium chloride (10%) was added. Then after an interval of 10 minutes, 1 ml of 1M sodium hydroxide and 1.2 ml of distilled water were added in the mixture. The mixture was vortexed and incubated for 10 minutes then the absorbance was read at 510 nm by spectrophotometer. A calibration curve was prepared using a standard solution of quercetin (0.05-0.5 mg/ml). Final results were expressed as mg quercetin equivalents/g (QE) of sample.

Vitamin C

Ascorbic acid was estimated by 2, 6 dichlorophenol indophenol titration method. Sample (10 g) was prepared in 3% (w/v) metaphosphoric acid and the volume was made upto 100 ml with metaphosphoric acid. Filtered aliquot (5 ml) of sample was titrated against standard 2, 6 dichlorophenol indophenol dye solution until the pink color developed completely.

Free radical scavenging activity using ABTS

A modified procedure using ABTS (2,2-azino-di- (3-ethylbenzothialozine-sulphonic acid) as described by Re *et al* was used. The ABTS+ stock solution (7 mM) was prepared through reaction of 7 mM ABTS and 2.45 mM

of potassium persulphate as the oxidizing agent. The working solution of ABTS+ was obtained by diluting the stock solution in ethanol to give an absorption of 0.70±0.02 at k =734 nm. Sample extracts (10 µl) were added to 90 µl of ABTS+ solution and absorbance readings at 734 nm were taken at 30ºC exactly 10 min after initial mixing. The percentage inhibition of ABTS+ of the test sample and known solutions of Trolox was calculated by the following formula: % inhibition =100·(A0- A)/A0 where A0 was the beginning absorbance at 734 nm, obtained by measuring the same volume of solvent, and A was the final absorbance of the test sample at 734 nm. The calibration curve between % inhibition and known solutions of Trolox (100–2000 µM) was then established. The radical-scavenging activity of the test samples was expressed as Trolox equivalent antioxidant capacity (TEAC µmol Trolox/g).

Statistical analysis

The tests were performed in triplicate, and the values were presented as means ± the standard deviations. Analysis of variance (ANOVA) and Duncan's method were carried out to relate significant differences between samples and solvents at the 95% level of confidence, using the SPSS Statistics 20 System for windows (SPSS Statistical Software, Inc., Chicago, IL, USA) software package.

Result and discussion

Total Phenolic Content

This method involves the oxidation/reduction on redox properties of antioxidant compounds present in the sample [Noriham *et al.*). The plant polyphenolics have strong potential to scavenge free radicals and therefore it is assumed that the antioxidant activities shown by plant materials occur due to presence of phenolic compounds (Skerget *et al.* 2005). The total phenolic content in different guava cultivars ranged from 4.18 to 34.39 mg GAE/gm. In all investigated Guava cvs, the phenolic content decreases as ripening proceeds (unripe >semiripe> ripe). The maximum content of phenolics was obtained in winter season followed by summer and rainy season in all the stages of ripening. Among the selected cultivars, Surkha has shown highest (34.39 mg/gm) phenolic content in winter season during unripe stage. There was no significant (P>0.05) difference between the cultivars Lalit, Chittidar and Safeda. In summer season the unripe stage of cultivar Red fleshed has given high value (23.96 mg/gm) of phenolics followed by Lalit (22.20 mg/gm) and Safeda (22.03 mg/gm). The phenolic content in rainy season was quite low as compare to other two seasons in unripe stage. The maximum value was seen for the cultivar Red fleshed (19.85 mg/gm) while the minimum phenolic content was found in cultivar Chittidar (13.89 mg/gm). The cultivars Surkha, Chittidar and Safeda has shown no significant difference in their phenolic content, while cultivars Lalit and Red fleshed significantly differ in their values. The results of semi-ripe stage showed that the maximum as well as minimum content of phenolics was found in cultivar Safeda (22.286 mg/gm), (4.186 mg/gm) in winter and rainy season respectively.

At the ripe stage of Guava fruit in winter season the phenolic content was found significantly (P>0.05) high in cultivar Lalit (11.640 mg/gm) and low value was recorded in cultivar Safeda (2.56 gm/gm). The seasonal pattern of increase and decline of phenolic content follows the same pattern as in unripe and semi-ripe stages (winter>summer>rainy).The amount of the antioxidant components that can be extracted is mainly affected by the vigour of the extraction procedure which probably may vary from sample to sample. The lack of fruit phenolic contents may be attributed to an amplified polyphenol oxidase activity as studied by Parr and Bolwell (2000) and secondly might have been due to loss in as trigingency during ripening as suggested by Taylor (1993), which may be caused by an increase in polymerization of leuco anthocyanidins and the hydrolysis of the astergingent arabinose ester of hexahydrodiphenic acid as seen by Stanislaw (1968). In agreement to our observations, a decreasing trend in the phenolic contents during ripening was also reported in high bush blueberries by Castrejon *et al.* (2003) and Kalt *et al.* (2008). Wang and Zhenge (2001) analysed that the variations of TPC in the guava fruits among different locations could be due to the influence of temperature and different prevalent environmental factors. The similar trend of decrease in phenolics with increase in ripening of fruit was also obtained by Gull *et al.* (2012) in Guava. Similar trend of total phenolics was obtained for summer and rainy season of ripe stage for all the selected cultivars. In the rainy season of ripe stage, phenolic content was found to be exceptionally low in all the selected cultivars. In red fleshed cultivars the percentage decline in winter season from unripe to semiripe 40%; semiripe to ripe 45%. Similar seasonal pattern of decline of phenolic content in unripe and semiripe stages was found in summer and rainy season in all the selected cultivars.

Flavonoid Content

Phenolic acids and flavonoids have been reported to be the main phytochemicals responsible for the antioxidant capacity of fruits and vegetables. Flavonoid content of the extracts was measured in terms of quercetin

equivalents. The results of flavonoid content in relation to fruit maturity/ripening stages decreases as the ripening increases (unripe>semiripe> ripe). The seasonal variation was same as in total phenolic content (winter > summer> rainy). The flavonoid content was in the range of 2.060 to 22.036 mg/gm. The mature fruit extract of white fleshed cultivar Safeda contains high amount of flavonoids (22.03 mg/gm) followed by red fleshed cvs Lalit (16.97 mg/gm), Surkha (15.38 mg/gm), Red Fleshed (15.66 mg/gm) and Chittidar (19.95 mg/gm). The maximum flavonoid content in summer season was obtained in white fleshed cultivars Safeda (22.03 mg/gm) and Chittidar (19.95 mg/gm). Among the red cultivars, the Red Fleshed cultivar has high content (15.66 mg/gm) than the other two cvs Lalit (12.98 mg/gm) and Surkha (12.02 mg/gm). Flavonoid content in rainy season was detected quite low as compare to other two seasons. In rainy season the red cultivars has shown fair amount of flavonoid content than white cultivars, red fleshed (11.38 mg/gm), Lalit (10.32 mg/gm), Surkha (10.11 mg/gm), Safeda (7.68 mg/gm) and chittidar (6.16 mg/gm). The total phenolics and flavonoids prevailed during the early maturity but their contents and antioxidant activity decreased with the advancing maturation as seen by Ho *et al.* in the leaves of *Psidium cattleianum*.

Vitamin C

Vitamin C, also known as ascorbic acid (AA), is considered as an enzymatic cofactor. It plays a vital role as an essential compound for plant tissues due to its considered antioxidant role. The table 3 showed the increasing trend of vitamin C with fruit ripening. The vitamin C content was in range of 17.240-125.556 mg/100gm. Maximum vitamin C content was found at ripe stage in every season. The highest amount was recorded in cultivar Safeda (125.66 mg/100gm) at the ripe stage in winter season followed by the cultivar Chittidar (118.49 mg/100gm), Lalit (114.47 mg/100gm), Surkha (111.566mg/100gm) and Red fleshed (98.473mg/100gm). Similar pattern of increase in vitamin C content was found for other selected cultivars in all the seasons of ripening stages. All the values differ significantly in winter season at ripe stage where as in summer and rainy season cultivars Surkha and Chittidar have no significant difference in their values and other cultivars differ significantly ($P<0.05$). The white cultivar Safeda has shown highest value in summer (103.306mg/100gm) as well as in rainy (94.413mg/100gm) season at ripe stage of ripening. At the semi-ripe stage, the maximum vitamin C was found in cultivar Safeda (93.396 mg/100gm) collected in winter season. Rainy season has shown low values in comparison to summer season at the semi-ripe stage, cultivar Chittidar (39.236 mg/100gm) recorded low value than other cultivars. The unripe stage of the fruit contains least amount of vitamin C in contrast to other stages as discussed above. The white fleshed fruit cultivar Safeda (50.673 mg/100gm) has high content of ascorbic acid in winter season where as the cultivar Red fleshed (17.240 mg/100gm) has low content of vitamin C collected in rainy season of unripe stage. The increase in vitamin C as the fruit matures is due to the breakdown of starch to glucose which is used in the biosynthesis of ascorbic acid. The similar trend of increase in vitamin C with increase in repining of fruit was also obtained by Gull *et al.* (2012) in Guava collected from various geographical areas. The Ilahy *et al.* (2011) and Tlili *et al.* (2011) also observed the same pattern of vitamin C in tomato cultivars and watermelon cultivars respectively. An increase in vitamin C content as the fruit matured (32 mg 100 g^{-1} to 144 mg 100 g^{-1} sample) was reported by Lim *et al*. Gomez and Lajolo exhibited 50% increase in vitamin C contents as a result of maturation in case of guava, but 35% decrease in vitamin C contents in case of mango during ripening. This inconsistent behavior of some fruits and different cultivars might be due to geographical and environmental conditions such as rain, temperature and soil. In fruits, variation in vitamin C contents due to several factors such as variety, species, cultivation practice and harvesting conditions has been reported. The other variables for example ambient temperature, relative humidity, oxidative stress, photosynthetic process, exposure of sun as well as pollutants are also considered as main contributors responsible for the variation in vitamin C contents. Different fruits exhibit different pattern of variation during storage and ripening processes. During the course of fruit ripening, vitamin C contents may decrease, increase or remain constant. The variability in the vitamin C content during repining of fruit was genotype dependent.

Scavenging power towards DPPH radical

The antioxidant activity measured by percent inhibition of DPPH by guava fruit extracts was shown in Table 4. The DPPH scavenging activity is a kinetic antioxidant method which based on the reduction of DPPH• free radical into $DPPH_2$ by the action of antioxidant. Ripening stages significantly affect the antioxidant activity of the fruits. The values of scavenging activity are in the range of 4.543-73.356%. The results obtained from the DPPH method of antioxidant activity determination showed that the unripe stages of the guava cultivars have maximum yield as compare to semi-ripe and ripe stage of maturity. The maximum radical scavenging activity was found in winter season followed by the fruit grown in summer

Table 1: Total Phenolic Content at different season and maturity stages on Guava cultivars

Stages	Season	Cultivars				
		Red fleshed	Lalit	Surkha	Chittidar	Safeda
Unripe	Winter	34.583 ± 0.877^{b}	33.640 ± 0.866^{a}	34.396 ± 1.016^{b}	31.356 ± 2.071^{a}	33.153 ± 2.017^{a}
	Summer	23.963 ± 1.265^{c}	22.203 ± 0.833^{b}	20.360 ± 0.775^{a}	19.956 ± 0.166^{a}	22.036 ± 0.580^{b}
	Rainy	19.856 ± 0.772^{c}	18.946 ± 0.737^{b}	14.113 ± 0.070^{a}	13.876 ± 0.110^{a}	14.380 ± 0.158^{a}
Semiripe	Winter	$20.880 \pm 1.325^{b,c}$	$20.486 \pm 0.806^{b,c}$	19.543 ± 0.567^{b}	17.173 ± 0.960^{a}	22.286 ± 1.830^{c}
	Summer	14.293 ± 0.954^{d}	13.070 ± 0.206^{c}	11.013 ± 0.332^{b}	9.983 ± 0.130^{a}	9.696 ± 0.299^{a}
	Rainy	11.776 ± 0.668^{d}	9.769 ± 0.212^{c}	7.436 ± 0.112^{b}	7.553 ± 0.575^{b}	5.346 ± 0.283^{a}
Ripe	Winter	11.420 ± 0.949^{b}	11.640 ± 0.935^{b}	11.326 ± 1.040^{b}	7.770 ± 0.326^{a}	9.183 ± 0.778^{a}
	Summer	6.863 ± 0.750^{c}	8.130 ± 0.130^{d}	5.860 ± 0.250^{b}	5.173 ± 0.547^{a}	4.186 ± 0.955^{a}
	Rainy	4.573 ± 0.430^{b}	4.580 ± 0.095^{b}	5.150 ± 0.145^{b}	4.860 ± 0.100^{b}	2.576 ± 0.685^{a}

All data are mean±SD of triplicate(n=3) analyses. Values with different superscript in the same column differ significantly ($p<0.05$).

Table2: Flavonoid content at different season and maturity stages on Guava cultivars

Stages	Season	Cultivars				
		Red fleshed	Lalit	Surkha	Chittidar	Safeda
Unripe	Winter	15.666 ± 0.934^{b}	16.973 ± 0.440^{c}	15.383 ± 0.781^{a}	19.956 ± 0.166^{d}	22.036 ± 0.580^{e}
	Summer	11.480 ± 0.256^{c}	7.236 ± 0.782^{b}	11.150 ± 0.079^{c}	5.273 ± 0.727^{a}	17.926 ± 0.987^{d}
	Rainy	5.273 ± 0.727^{b}	5.150 ± 0.101^{b}	10.110 ± 0.100^{d}	2.486 ± 0.386^{a}	7.686 ± 0.105^{c}
Semiripe	Winter	12.170 ± 1.028^{a}	15.550 ± 0.821^{c}	13.246 ± 0.956^{b}	12.886 ± 0.895^{a}	20.393 ± 0.757^{d}
	Summer	11.293 ± 1.100^{b}	11.943 ± 0.281^{c}	12.020 ± 0.026^{d}	9.983 ± 0.130^{a}	9.696 ± 0.299^{a}
	Rainy	10.153 ± 0.172^{d}	9.036 ± 0.075^{c}	9.610 ± 0.186^{c}	5.233 ± 0.102^{a}	6.120 ± 0.130^{b}
Ripe	Winter	12.886 ± 0.895^{d}	12.983 ± 0.206^{d}	9.403 ± 1.136^{a}	11.293 ± 1.101^{b}	12.490 ± 0.902^{c}
	Summer	5.823 ± 0.422^{b}	10.326 ± 1.132^{d}	8.053 ± 0.310^{c}	5.173 ± 0.547^{a}	4.186 ± 0.955^{a}
	Rainy	3.360 ± 0.329^{b}	10.326 ± 0.097^{e}	4.323 ± 0.240^{c}	6.166 ± 0.165^{d}	2.060 ± 0.050^{a}

All data are mean±SD of triplicate(n=3) analyses. Values with different superscript in the same column differ significantly ($p<0.05$).

Table 3: Vitamin C values at different season and maturity stages on Guava cultivars

Stages	Season	Cultivars				
		Red fleshed	Lalit	Surkha	Chittidar	Safeda
Unripe	Winter	22.293 ± 0.903^{a}	46.450 ± 1.035^{c}	44.540 ± 0.629^{b}	43.606 ± 0.977^{b}	50.673 ± 0.491^{d}
	Summer	18.523 ± 0.615^{a}	34.263 ± 0.905^{c}	29.936 ± 0.275^{b}	28.983 ± 0.184^{b}	35.510 ± 0.560^{d}
	Rainy	17.240 ± 0.120^{a}	30.286 ± 0.909^{d}	25.726 ± 0.751^{c}	23.153 ± 1.027^{b}	26.473 ± 0.755^{c}
Semiripe	Winter	60.180 ± 1.065^{a}	85.510 ± 0.742^{c}	81.183 ± 0.997^{b}	86.343 ± 1.194^{c}	93.396 ± 0.847^{d}
	Summer	50.086 ± 0.842^{b}	68.093 ± 0.568^{c}	50.426 ± 0.708^{b}	48.026 ± 0.135^{a}	69.336 ± 0.573^{d}
	Rainy	48.183 ± 0.532^{c}	64.373 ± 1.085^{d}	46.410 ± 0.697^{b}	39.236 ± 0.741^{a}	45.266 ± 0.885^{b}
Ripe	Winter	98.473 ± 1.133^{a}	114.473 ± 0.775^{c}	111.566 ± 1.236^{b}	118.496 ± 1.189^{a}	125.556 ± 0.905^{e}
	Summer	82.973 ± 0.181^{b}	88.763 ± 0.922^{c}	80.360 ± 0.441^{a}	83.660 ± 0.501^{b}	103.306 ± 0.883^{d}
	Rainy	79.326 ± 0.785^{c}	86.413 ± 1.153^{d}	68.653 ± 1.246^{a}	71.723 ± 0.414^{b}	94.413 ± 1.115^{e}

All data are mean±SD of triplicate(n=3) analyses. Values with different superscript in the same column differ significantly ($p<0.05$).

and rainy season. The highest percent scavenging activity was found in red cultivar Lalit (73.35 %) at unripe stage in winter season, followed by cultivar Safeda (66.17%), Surkha (63.22%), Chittidar (60.36%) and Red fleshed (58.18%). Cultivar Lalit (33.25%) had shown higher scavenging activity in summer and rainy season too, as compare to other cultivars Surkha (32.36 %), Chittidar (30.38%), Safeda (30.82%) and Red fleshed (19.39%) in summer season, and Chittidar (20.43%), Safeda (19.64%), Surkha (19.28%) and Red fleshed (18.59) in rainy season. The semiripe stage results in gradual decline of free radical scavenging activity as compare to unripe

Table 4: DPPH values at different season and maturity stages on Guava cultivars

Stages	Season	Cultivars				
		Red fleshed	Lalit	Surkha	Chittidar	Safeda
Unripe	Winter	58.1833±0.996^{a}	73.3567±1.060^{e}	63.226±0.755^{c}	60.3667±0.755^{b}	66.1767±0.746^{d}
	Summer	19.3967±1.020^{a}	33.2567±1.151^{e}	32.360±1.096^{c,d}	30.3833±0.911^{b}	30.8267±0746^{b,c}
	Rainy	18.596±0.889^{a}	22.4833±0.662^{d}	19.283±0.941^{a,b}	20.4300±0.670^{c}	19.6467±1.022^{a,b}
Semiripe	Winter	49.3300±0.914^{a}	50.3367±0.7814^{a}	55.376±1.062^{b}	49.1200±1.031^{a}	56.6233±0.528^{b}
	Summer	14.8133±0.557^{a}	21.4500±0.871^{c}	21.850±1.050^{c}	19.5767±0.482^{b}	22.0700±0.528^{c}
	Rainy	10.283±0.842^{a}	13.5933±0.796^{b}	12.680±0.721^{b}	13.2067±0.721^{b}	13.4500±1.179^{b}
Ripe	Winter	30.3833±1.071^{c}	42.3600±0.7518^{d}	23.360±0.975^{a}	28.3467±1.108^{b}	23.0867±0.972^{a}
	Summer	7.5067±0.740^{a}	14.2067±1.101^{b}	12.726±1.011^{b}	14.4100±1.210^{b}	13.9133±0.940^{b}
	Rainy	5.333±0.618^{a}	7.2667±0.869^{b}	4.880±0.838^{a}	7.5633±1.151^{b}	4.5433±0.988^{a}

All data are mean±SD of triplicate(n=3) analyses. Values with different superscript in the same column differ significantly ($p<0.05$).

Table 5: FRAP values at different season and maturity stages on Guava cultivars

Stages	Season	Cultivars				
		Red fleshed	Lalit	Surkha	Chittidar	Safeda
Unripe	Winter	35.6500±0.841^{b}	39.1800±0.0556^{c}	22.7067±0.617^{a}	22.0367±0.990^{a}	21.3200±0.775^{a}
	Summer	25.4700±0.731^{d}	23.2400±1.105^{c}	17.4700±0.773^{b}	15.2700±0.876^{a}	14.4433±0.775^{a}
	Rainy	21.393±1.094^{c}	17.4933±0.998^{b}	17.0100±0.670^{b}	11.4200±0.670^{a}	11.1967±0.846^{a}
Semiripe	Winter	23.4700±0.791^{d}	21.6900±0.050^{c}	14.216±0.977^{b}	15.4100±1.249^{b}	10.2867±0.4046^{a}
	Summer	20.1033±0.786^{d}	18.3933±1.070^{c}	14.1000±1.005^{b}	12.7133±0.962^{a,b}	12.2433±0.404^{a}
	Rainy	15.398±0.646^{c}	16.7833±0.570^{c}	12.4200±1.191^{b}	5.5067±1.191^{a}	5.3033±0.859^{a}
Ripe	Winter	13.1800±0.990^{d}	12.6467±0.049^{c}	8.653±0.702^{b}	6.5267±0.868^{a}	7.7133±1.231^{a,b}
	Summer	10.4833±0.520^{b}	10.5067±0.515^{b}	6.6933±0.948^{a}	6.7133±0.890^{a}	6.6600±0.846^{a}
	Rainy	8.203±0.946^{b}	10.3967±0.747^{c}	7.4800±0.598^{b}	2.3033±1.123^{a}	1.3700±0.680^{a}

All data are mean±SD of triplicate(n=3) analyses. Values with different superscript in the same column differ significantly ($p<0.05$).

Table 6: ABTS values at different season and maturity stages on Guava cultivars

Stages	Season	Cultivars				
		Red fleshed	Lalit	Surkha	Chittidar	Safeda
Unripe	Winter	20.3367±1.026^{c}	19.2633±0.921^{c}	16.153±1.062^{b}	14.7600±0.422^{b}	13.2833±0.975^{a}
	Summer	17.1967±1.176^{c}	14.2733±0.631^{a}	13.6867±1.415^{a}	12.5267±1.278^{a}	15.2900±0.975^{b}
	Rainy	12.253±0.781^{b}	9.3300±1.073^{a,b}	10.1400±0.982^{b}	8.3100±0.982^{a}	10.1700±0.897^{b}
Semiripe	Winter	16.3167±1.154^{d}	14.1967±0.920^{c}	7.483±1.292^{b}	5.6300±0.652^{a}	4.3200±0.858^{a}
	Summer	10.3167±0.678^{d}	8.6167±0.814^{c}	7.6933±0.504^{b}	4.4900±1.0725^{a}	11.3567±0.769^{c}
	Rainy	8.486±1.128^{c}	4.4900±0.883^{b}	3.4433±0.625^{b}	1.6733±0.549^{a}	4.3433±1.086^{b}
Ripe	Winter	8.2467±0.915^{b}	8.3000±0.930^{b}	2.150±0.895^{a}	1.9267±0.228^{a}	1.2633±0.741^{a}
	Summer	5.0500±0.427^{b}	3.9533±0.208^{b}	4.3600±1.028^{b}	1.3233±0.582^{a}	2.5433±0.741^{a}
	Rainy	1.433±0.779^{a}	1.1800±0.334^{a}	1.0167±0.435^{a}	1.3733±0.435^{a}	0.7433±0.481^{a}

All data are mean±SD of triplicate(n=3) analyses. Values with different superscript in the same column differ significantly ($p<0.05$).

stage. In this stage cultivar Safeda (56.62%) and Surkha (55.37%) had shown high amount of antioxidant activity at in winter season with respect to other cultivars. The biggest significant difference ($P < 0.05$) in the scavenging activity was obtained in fruit from unripe to ripe stage. In ripe stage, cultivar Lalit (42.36%) has higher radical scavenging acivity in winter season. The trend of cultivars in winter season was Red fleshed (30.38%), Chittidar (28.34%), Surkha (23.36) and Safeda (23.08%). The values in the ripe stage varied significantly. The values in summer and rainy season followed the same trend of cultivars as in winter season. The similar pattern of decline in DPPH activity was reported by Manach *et al*., in sample of *Capsicum chinese Habanero* showed

Table 7: Pearson's correlation coefficients between total phenolics, flavonoids ,vitamin C and DPPH, FRAP, ABTS assay of winter season at various maturity stages in Guava cultivars

	DPPH	FRAP	ABTS
TPC	0.905641309	0.824187092	0.83196971
FLAVONOID	0.730363821	0.675935783	0.680987955
VITAMIN C	-0.835135112	-0.872639088	-0.898054214

Table 8: Pearson's correlation coefficients between total phenolics, flavonoids ,vitamin C and DPPH, FRAP, ABTS assay of summer season at various maturity stages in Guava cultivars

	DPPH	FRAP	ABTS
TPC	0.787209676	0.841192588	0.930747542
FLAVONOID	0.618517439	0.787909444	0.868643852
VITAMIN C	-0.745564885	-0.797968437	-0.8563628

Table 9: Pearson's correlation coefficients between total phenolics, flavonoids ,vitamin C and DPPH, FRAP, ABTS assay of rainy season at various maturity stages in Guava cultivars

	DPPH	FRAP	ABTS
TPC	0.872418327	0.826916528	0.925448451
FLAVONOID	0.710181948	0.918436848	0.797605181
VITAMIN C	-0.904102611	-0.605795742	-0.841776239

the highest DPPH activity at the younger stage while at the older stage the activity was lower.

Ferric Reducing Antioxidant Activity

The antioxidant capacity of fruit extracts is based on the ability of the antioxidants to reduce ferric iron to ferrous in FRAP reagent. The reduction of ferric iron in FRAP reagent will result in the formation of a blue product (ferrous-TPTZ complex) that can be detected at absorbance 593 nm. In contrast to other tests of total antioxidant activity, the FRAP assay is simple, speedy, inexpensive, and highly reproducible. FRAP values for every season with maturity stages was in the range of 39.18- 1.37 mM Fe(II)/gm. The maximum FRAP content was recorded in sequence of winter>summer>rainy and unripe>semi-ripe>ripe with respect to season and maturity stages respectively. At unripe stage, cultivar Lalit (39.18 mM Fe(II)/gm) found to possess high amount of ferric reducing power in contrast to other cultivars *i.e.*, Red fleshed (35.65 mM Fe(II)/gm), Surkha (22.70 mM Fe(II)/gm), Chittidar (22.03 mM Fe(II)/gm) and Safeda (21.32 mM Fe(II)/gm) in winter season. Where as in summer and rainy season, the cultivar Red fleshed (25.47 mM Fe(II)/gm) was recorded to have maximum amount of FRAP values in comparison to other cultivars. At semiripe stage, the Red Fleshed cultivar had high ferric reducing power as compare to other cultivars in all seasons. The value recorded in winter season was maximum (23.47 mM Fe(II)/gm) followed by summer (20.10 mM Fe(II)/gm) and rainy season (15.39 mM Fe(II)/gm). The ripe stage had shown lower values of FRAP antioxidant activity among other satges of maturity. Cultivar Red fleshed (13.18 mM Fe(II)/gm) and Lalit (12.64 mM Fe(II)/gm) possesses high values in every seasons. The similar result was observed by Ding *et al.* on the pineapple fruit, they concluded that the antioxidant activity using FRAP assay showed an increase from mature green stage to 50% yellow stage and decrease from 75% yellow to 100% yellow. The reduction in FRAP values during guava fruit maturation may be associated with the relative decrease in content of various polyphenol compounds that constitute the total phenolic content in the fruit as per Fischer *et al*.

$ABTS^+$ free radical scavenging activity

ABTS assay is based on the reaction of the ABTSÿ+ radical cation generated in the assay with the antioxidant present in the sample. This method takes comparatively less time than the other methods and it is also used to confirm the result obtained with DPPH, as both are similar in their antioxidant mechanism. The $ABTS^+$ free radical scavenging activity of Guava fruit varied significantly among the cultivars and during stages of ripening. The values ranged between 20.33 to 0.74 μmol Trolox/g. This activity showed inverse relation with maturity stages in all the cultivars with every season. At the unripe stage, cultivar Red fleshed (20.33 μμολ Τρολοξ/g) had shown the highest cation activity in winter season as compare to other cultivars Lalit (19.26 μmol Trolox/g), Surkha (16.15 μμολ Τρολοξ/g), Chittidar (14.76 μμολ Τρολοξ/g) and Safeda (13.28 μmol Trolox/g). At semiripe stage also the cultivar Red Fleshed (16.31 μmol Trolox/g) had given the high values in winter season followed by summer and rainy seasons. Other cultivars reported to have low values like cultivar Lalit (14.19 μmol Trolox/g), Surkha (7.48 μμολ Τρολοξ/g), Chittidar (5.63 μμολ Τρολοξ/g) and Safeda (4.32 μμολ Τρολοξ/g). The similar decreasing trend was observed in ripe stage of the guava fruit. The lowest values of ABTS activity was observed in cultivar Safeda (0.74 μμολ Τρολοξ/g) in rainy season. Chodak *et al.* (2011) had shown that the capacity to scavenge an ABTS radical in apples decreased during ripening.

Correlation Studies

Correlation analysis was done to explore the relationships amongst the different antioxidant assays

measured for guava cultivars collected in different seasons. The results of total phenolics, flavonoid, vitamin C, DPPH, FRAP and ABTS assay used in the present work were compared and correlated. The correlation of total phenolics, flavonoid and vitamin C of guava cultivars with DPPH, FRAP and ABTS activity at different season was shown in table 7, 8 and 9 respectively. In winter season the correlation coefficient was higher (R^2=0.905) between total phenolics and DPPH activity than that of FRAP activity (R^2=0.824) and ABTS activity (R^2 =0.832). The correlation of flavonoid was also found high with DPPH activity (R^2=0.703) as compare to FRAP (R^2=0.680) and ABTS activity (R^2=0.675). The negative correlation was seen in vitamin C with DPPH, FRAP and ABTS activity. The correlation coefficient in summer season was found maximum in total phenolics and ABTS activity (R^2=0.930) as compare to FRAP (R^2=0.841) and DPPH activity (R^2=0.787). The same correlation was observed with flavonoid *i.e.*, maximum with ABTS (R^2=0.868) than FRAP (R^2=0.787) and DPPH activity (R^2=0.618). In summer season also the negative correlation was seen in vitamin C with DPPH, FRAP and ABTS activity. In rainy season the correlation coefficient was greater in total phenolics and ABTS activity (R^2=0.925) followed by DPPH (R^2=0.872) and FRAP activity (R^2=0.826). The strong correlation coefficient was seen among flavonoid and FRAP (R^2=0.918) than ABTS (R^2=0.797) and FRAP activity (R^2=0.710). The vitamin C was again shown the negative correlation coefficient with the antioxidant activities (DPPH, FRAP and ABTS). The antioxidant capacity of the fruit appears to be largely influenced by polyphenolic and flavonoid levels. A highly significant linear correlation was observed between antioxidant capacity of the extracts of the fruit in various seasons and their phenolic and flavonoid content. The ethanolic extract of guava cultivars possessed the ability to quench the reactive oxygen species or free radicals. The findings from the above correlation analyses indicated specific phenolic substances, which were extracted by the selected cultivars and seasons, had different degrees of contributions to the overall antioxidant activities. These results are in accordance with many others where it is shown that higher total phenolic linearly correlates well with antioxidant activity (Viraj and Pillai 2012).

References

Ahmad, N., F. Anwar, S. Hameed and M.C. Boyce (2011). Antioxidant and antimicrobial attributes of different solvent extracts from leaves and flowers of akk (*Calotripis procera* (Ait.) Ait. F). *Journal of Medicinal Plants Research*, **5(19)**: 4879-4887.

Alothman, M., R. Bhat and A.A. Karim (2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chemistry,* **115**: 785–788.

Boateng, J., M. Verghese, L.T. Walker, S. Ogutu (2008). Effect of processing on antioxidant contents in selected dry beans (*Phaseolus* spp. L.). *LWT - Food Science and Technology,* **41**: 1541-1547

Castrejon, A.D.R., I. Eichholz, S. Rohn, L.W. Kroh and S. Huyskens-Keil (2008). Phenolic profile and antioxidant activity of highbush blueberry (*Vacciniumcorymbosum* L.) during fruit maturation and ripening. *Food Chem.*, **109**: 564–572.

Cordenunsi, B.R., J.R.O. Nascimento, M.I. Genovese and F.M. Lajolo (2002). Influence of cultivar on quality parameters and chemical composition of strawberry fruits grown in Brazil. *J. Agric. Food Chem,* **50**: 2581–2586.

Ding, P. and S. Syazwani (2015). Maturity stages affect antioxidant activity of 'md2' pineapple (*Ananas comosus* l.), 10.17660/*Acta Hortic.*, 1088.34

Fischer, U.A., R. Carle, D.R. Kammerer (2011). Identification and quantification of phenolic compounds from pomegranate (*Punica granatum* L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD–ESI/MSn. *Food Chemistry,* **127**: 807–821.

Girard-Lalancette, K., A. Pichette and J. Legault (2009). Sensitive cell-based assay using DCFH oxidation for the determination of pro- and antioxidant properties of compounds and mixtures: Analysis of fruit and vegetable juices. *Food Chemistry,* **115**: 720-726.

Gomez, M.L.P.A. and F.M. Lajolo (2008). Ascorbic acid metabolism in fruits: Activity of enzymes involved in synthesis and degradation during ripening in mango and guava. *J. Sci. Food Agric.,* **88**: 756–762.

Ho, R., A. Violette, D. Cressend, P. Raharivelomanana, P.A. Carrupt and K. Hostettmann (2012). Antioxidant potential and radical-scavenging effects of flavonoids from the leaves of *Psidium cattleianum* grown in French Polynesia. *Natural Product Research,* **26(3)**: 274–277.

Joseph, B. and R.M. Priya (2011). Phytochemical and Biopharmaceutical Aspects of *Psidium guajava* (L.) Essential Oil: A Review. *Research Journal of Medicinal Plants,* **5**:432-442.

Kalt, W., C. Lawand, D. Ryan, J.E. McDonald and H. Donner (2003). Oxygen radical absorbing capacity, anthocyanin and phenolic content of highbush blueberries (*Vacciniumcorymbosum* L.), during ripening and storage. *J. Am. Soc. Hortric. Sci.*, **128**: 917–923.

Noriham, A., S.S.W.K. Wan, S. Zainal, S.Z. Khairusy and A. Nurain (2012). Study on antioxidant capacity and phenolic

content of various parts of wax gourd (*Benincasahispida*). *World Applied Science Journal,* **19(7)**: 1051–1056.

Parr, A.J. and P.A.J. Bolwell (2000). Phenols in the plant and in man: The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *J. Sci. Food Agric.*, **80**: 985–1012.

Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, *et al*. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol. Med.,* **26**: 1231-1237.

Renaud, S.C., R. Gueguen, J. Schenker and A. d'Houtaud (1998). Alcohol and mortality in middle-aged men from eastern France. *Epidemiology,* **9**: 184 – 188.

Riadh, I., C. Hdider, S.L. Marcello, I. Tlilia and G. Dalessandro (2011). Antioxidant activity and bioactive compound changes during fruit ripening of high- lycopene tomato cultivars. *Journal of Food Composition and Analysis,* **24**: 588–595

Skerget, M., P. Kotnik, M. Hadolin, A.R. Hras, M. Simonic and Z. KnezPhenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities.

Stanislaw, L. (1968). Determination of the degree of polymerization of leucoanthocyanidins. *Phytochemistry*, **7**: 665–667.

Tlilia, I., I. Riadh, C. Hdider, S.L. Marcello and G. Dalessandro (2011). Bioactive compounds and antioxidant activities during fruit ripening of watermelon cultivars. *Journal of Food Composition and Analysis,* **24**: 923–928.

Van't Veer, P., M. Janson, M. Klert and F. Kok (2000). Fruits nd vegetables in the prevention of cancer and cardiovascular disease. Public Health Nutrition, **3**: 103-107.

Viraj and Pillai (2012). Phenolic content and antibacterial effect of guava (*Allahabad safeda* and *Bhavnagar red*). *Journal of Cell and Tissue Research,* **12(2)**: 3255-3260.

Wang, S.Y.; W. Zheng (2001). Effect of plant growth temperature on antioxidant capacity in strawberry., *J. Agric. Food Chem.,* **49**: 4977–4982.

Williamson, G. and C. Manach (2005). Bioavailability and bioefficacy of polyphenols in humans II. Review of 93 intervention studies. *Am. J. Clin. Nutr.*, **81**: 243S-255S.