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Effect of stage of maturity, ripening and storage on antioxidant content and activity of *Mangifera indica L. var.* Manjira

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Abstract
The present study was carried out to appraise the antioxidant potential of Manjira variety of mango in which comparison of two stages of maturity (commercial maturity: 7 to 9°brix and physiological maturity: 9 – 11°brix) of the mango cultivar and three different ripening processes (control ripening, 100ppm ethylene ripening and 150ppm ethylene ripening) was done. The antioxidant activity of the mango was measured through various in vitro assays such as total antioxidant activity, reducing power, DPPH, FRAP, superoxide radical scavenging activity and H_2O_2 scavenging activity. Overall, fruit without any ethylene treatment exhibited highest reducing power, H_2O_2 scavenging activity and DPPH activity, followed by 100ppm ethylene treated mangoes which had highest total antioxidant activity, superoxide radical scavenging activity, FRAP. 150ppm ethylene treated mangoes also had good reducing power, superoxide radical scavenging activity and DPPH activity but the fruits were very soft on 12th day of storage and could not be stored beyond. Control mangoes and 100ppm ethylene treated mangoes which were harvested at 7 to 9°brix maturity stage had higher antioxidant values indicating that mangoes when harvested at 7 to 9°brix maturity stage would provide high antioxidant potential.

Keywords: mango, ripening, ethylene, maturity stage, antioxidant

1. Introduction
Mango (*Mangifera indica L.*) is one of the most nutritious tropical fruits, native to southern Asia and especially Eastern India. The mango tree is from the Anacardiaceae family and it was disseminated all over the world in the beginning of the sixteenth century, and there are currently around a thousand known varieties of mango [1]. Mango is the dominant tropical fruit variety produced worldwide, followed by pineapples, papaya and avocado, all of which are considered major tropical fruits. The global production of mango in 2010 was estimated to be about 35 million tons, accounting for nearly 50% of world tropical fruit production [2].

Fruit ripening is a highly coordinated, genetically programmed and irreversible phenomenon involving a series of biochemical and organoleptic changes that lead to the development of a soft and edible ripe fruit with desirable quality attributes [3]. During ripening, fruit undergo a series of biochemical, physiological and structural changes which make them attractive to the consumer [4].

Mango is an excellent source of bioactive compounds such as provitamin A carotenoids, vitamin C and phenolics, as well as dietary fibre [5, 6, 7, 8], essential to human nutrition and health.Mango is rich in a variety of phytochemicals and nutrients that qualify it as a model "superfruit", a term used to highlight potential health value of certain edible fruits. The fruit is high in prebiotic dietary fiber, vitamin C, polyphenols, amino acids, carotenoids, minerals and antioxidants. Carotenoids are known to be unique constituents of a healthy diet and have been associated with reducing the risk of several degenerative disorders, including various types of cancer, cardiovascular or ophthalmological diseases [9]. Mango contains high concentrations of phytochemicals, including gallic acid, mono galloylglucosides, gallotannins, flavonol glycosides and benzophenone derivatives [10], some of which are unique to the plant and have been proposed for use in creating phytochemical rich dietary supplements [11]. In addition, gallotannins have exhibited antioxidant and anti-inflammatory properties [12]. However, the effectiveness of mango polyphenolics may vary according to the types and contents of the specific compounds, such as gallic acid, galloyl-glucosides, and gallotannins. The polyphenolic contents of mango are influenced by varieties, ripening, cultivar, environmental, and handling factors [13]. In the human body, phenols and flavonoids relate to bioactive compounds involving a decrement of differing deteriorative processes. It is because of its capacity to lessen free radical build up as well as to scavenge free radicals [14]. Phenolic compounds are important bioactives and their content in fruits represents an important fruit quality parameter [15]. Phenolics, as a well-known class of plant secondary metabolites, are effective free radical scavengers and also show multiple medicinal and biological functions in animals as well as in plant [16]. In

general, the antiradical and antioxidant activities of plant extracts are associated to the phenolic content [17].Fruits and vegetables are rich source of flavonoids [18, 19]. Carotenoids like β-carotene and lycopene present in plants exert antioxidant functions such as quenching of singlet oxygen and other electronically excited molecules and progression of many degenerative diseases [20, 21].

Evaluation of antioxidant activity is becoming increasingly relevant in the field of nutrition as it provides useful information with regard to health promoting and functional quality of raw material [22]. The parameter accounts for the presence of efficient oxygen radical scavengers, such as vitamin C, carotenoids, flavonoids and phenolic compounds and their synergistic and/or antagonistic effects. There is no precise information on the exact stage of harvest and effect of ripening treatments on the antioxidant content and antioxidant activity of mangoes. New knowledge about mango consumption is relevant to rational development in knowing the right stage for mango consumption with highest antioxidant potential. In view of this, a study was taken up to investigate effect of stage of maturity, ripening and storage on antioxidant activity of (*Mangifera indica L). Var.* Manjira.

2. Materials and method

2.1 Sample Collection and treatment

Sixty mature green undamaged and healthy fruits of the Manjira cultivar were harvested at two maturity stages (7-9°brix TSS and 9-11°brix TSS) at Fruit Research Station, SKLTSHU, Sangareddy. The required number of unblemished physically similar, more or less uniform size, shape and color fruits for the experiment were harvested manually from the tree. The fruits were carefully selected during harvest. After harvest, the fruits were allowed for desapping for one hour and then the skin of fruits was cleaned with the help of a cloth followed by washing the fruits with mild neutral detergent sandovit (0.5ml/litre). The fruits were then shade dried and taken for ethylene treatment. Two ethylene ripening treatments (100ppm & 150ppm ethylene doses) were carried out at Fruit Research Station, SKLTSHU, Sangareddy.

2.2 Sample Extraction

For the assessment of Vitamin C and Total Carotene content fresh mango pulp was used whereas for estimation of Total phenols, Total Flavonoids, Total Antioxidant activity, DPPH, Ferric Reducing power assay, Superoixide radical scavenging activity, H_2O_2 Scavenging activity and Reducing power ethanolic extract was used. Five grams of dehydrated pulp was extracted twice with 30ml of ethanol (80%), by stirring and sonicating for 30 min in dark. The homogenate was then centrifuged for 15 min at 10,000rpm at 4˚C (Eppendorf, Westbury, USA). The supernatant was then vacuum concentrated at 40ºC in a rota-evaporator and stored at -20ºC until further analysis. During the estimation, if the absorbance was higher than the standard, further dilution was made with respective solvents for the assay. The results of Vitamin C, Ferric Reducing Antioxidant Power, Superoxide radical Scavenging activity, DPPH, Reducing Power, Total Antixoidant activity were expressed in Ascorbic acid Equivalence, Total Flavonoids in Quercetin Equivalence, and Total Phenols in Gallic acid Equivalence.

2.3 Antioxidant profile

2.3.1 Determination of Total carotenes

The total carotene content was determined using method described by Zakaria *et al*. [23] and results were expressed µg/100g. 0.5 g sample was homogenized and saponified with 2.5 ml of 12% alcoholic potassium hydroxide in a water bath at 60°C for 30 minutes. After which the extract was transferred to a separating funnel containing 10 – 15 ml of petroleum ether and mixed well. The lower aqueous layer was then transferred to another separating funnel and the upper petroleum ether layer containing the carotenoids was collected. The extraction was repeated until the aqueous layer became colourless. A small amount of anhydrous sodium sulphate was added to the petroleum ether extract to remove excess moisture. The final volume of the petroleum ether extract was noted and absorbance was measured in a spectrophotometer at 450 nm using petroleum ether as blank.

2.3.2 Determination of Vitamin C

Ascorbic acid in mango pulp was estimated by 2, 6-Dichlorophgenol-induphenol tirtation method as described by AOAC. 2 to 3 g of mango flesh was cut into small pieces and homogenized well with 20 ml of 3% metaphosphoric acid and filtered through double layers of muslin cloth. The filtrate was centrifuged at 3,000 x g for 10min and the clear supernatant was titrated with 2, 6-dichlorophenol indophenol solution. Ascorbic acid was used as a standrard and results were expressed as mg per 100g.

2.3.3 Determination of Total Flavonoid

The total flavonoid content was estimated by aluminium chloride colorimetric method described by Ling and Tang [24]. 0.1 g of mango extract was dissolved in 1 ml deionized water and then 0.5 ml of this solution was mixed with 1.5 ml of 95% alcohol, 0.1 ml of 10% aluminium chloride hexahydrate ($AlCl_3$), 0.1 ml of 1 M potassium acetate (CH_3COOK), and 2.8 ml of deionized water. Then it was incubated at room temperature for 40 min and absorbance was measured at 415nm against deionized water blank on a spectrophotometer. Quercetin was chosen as a standard. Using a seven point standard curve (0–50 mg/l), the levels of total flavonoid contents in mango extract were determined in triplicate, respectively. Results were expressed in milligram quercetin equivalents (QE)/100g fresh matter from mango based on the moisture content of mango.

2.3.4 Determination of Total phenols

Total phenolic contents of mango extract were determined by Folin-Ciocalteu method [24] and results were expressed as milligram gallic acid equivalents (GAE)/100g. 0.1 g mango extract were dissolved in 1 ml deionized water and the 0.1 ml of this solution was mixed with 2.8 ml of deionized water, 2 ml of 2% sodium carbonate (Na_2CO_3), and 0.1 ml of 50% Folin-Ciocalteau reagent. The absorbance of the sample was measured at 750 nm against deionized water blank on a spectrophotometer after incubation at room temperature for 30 min. Gallic acid (GA) was chosen as a standard. Using a seven point standard curve (0–200 mg/l), the levels of total phenolic contents in mango extract were determined in triplicate, respectively.

2.4 Antioxidant activity of mango

2.4.1 Determination of Total antioxidant capacity

Total antioxidant capacity was assessed using a modified method as described by Damiki and Alakh [25]. 0.3 ml mango pulp extract was combined with a mixture of 3 ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were then capped and incubated at 95°C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the solution was then measured at 695 nm against blank. Methanol (0.3 ml) in the place of extract was used as the blank. The antioxidant activity was expressed as the mg of equivalents of ascorbic acid.

2.4.2 Determination of Hydrogen peroxide scavenging activity

Ruch *et al*. method was used to assess the ability of extracts to scavenge hydrogen peroxide [26]. Ascorbic acid solutions (200 mg/l in methanol) were used as comparative standard molecules. Hydrogen peroxide solution (2 mM/l) was prepared in phosphate-buffered saline (PBS, pH 7.4). Hydrogen peroxide (H_2O_2) concentration was determined spectrophotometrically from absorption at 230 nm with the molar absorptivity of 81 $(M/l^{-1})^{-1}.cm^{-1}$. One milliliter of mango extract was added to H_2O_2 solution (0.6 ml) and absorbance of the hydrogen peroxide at 230 nm was read after 10 min against a blank solution containing extract (1 ml) in PBS without H_2O_2. The scavenging of hydrogen peroxide was determined as using the following formula:

$$\%Svg = \left[\frac{A_m}{A_b}\right] \times 100$$

Where A_m is Absorbance of reaction mixture and A_b is Absorbance of blank mixture (extract in PBS without H_2O_2).

2.4.3 Superoxide scavenging activity

The superoxide scavenging activity was evaluated using NBT reduction method [27]. The reaction was started by adding 100 µl of phenazine methosulfate solution (60µM,PMS) in phosphate buffer (pH 7.4) to the reaction mixture consisting of 1 ml of NBT(156 µM) and sample solution, followed by incubation at 25˚C for 5 min and the absorbance at 560 nm was measured against blank. Ascorbic acid was used as standard.

$$\text{Superoxide scavenging activity (\%)} = \frac{Abs(control) - Abs(Sample)}{Abs(control)} \times 100$$

2.4.4 Reducing power assay

The reducing power of the extract was determined according to the method of Oyaizu [28]. The aliquots of various concentrations of the standard and mango pulp extracts (10 to 100 µg/ml) was added to 1.0 ml of deionized water and mixed with 2.5 ml of (pH 6.6) phosphate buffer and 2.5 ml of (1%) potassium ferricyanide. The mixture was incubated at 50°C in water bath for 20 min after cooling. Aliquots of 2.5 ml of (10%) tri-chloro-acetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of 2.5 ml solution was mixed with 2.5 ml distilled water and a freshly prepared 0.5 ml of (0.1%) ferric chloride solution. The absorbance was measured at 700 nm in UV spectrometer. A blank was prepared without adding extract. Ascorbic acid at various concentrations (10 to 100 µg/ml) was used as standard.

2.4.5 DPPH radical scavenging activity

The free radical scavenging capacity of the extracts was determined using 1, 1- dipheny l- 2-picryl-hydrazil (DPPH)[29] and ascorbic acid was used as standard. 2 ml of methanol solution of DPPH radical in the concentration of 0.05 mg/ml and 1ml of mango pulp extract were placed in cuvettes. The mixture was shaken and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm against methanol as blank in spectrophotometer. The DPPH free radical concentration was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = (A_0 - A_1 / A_0) \text{ X } 100$$

Where A_0 is the absorbance of the negative control and A_1 is the absorbance of reaction mixture or standards.

2.4.6 Ferric reducing antioxidant potential assay (FRAP)

Ferric reducing antioxidant potential (FRAP) of the mango extract was measured according to the method proposed by [30]. FRAP reagent was prepared by mixing in 25 ml acetate buffer (30 mM; pH 3.6), 2.5 ml TPTZ solution (10 mM) and 2.5ml ferric chloride solution (20 mM). The mixture was incubated for 15 min at 37°C before use. Ascorbic acid (vitamin C) was employed as a standard in this assay, and its calibration curve was obtained by using its concentrations ranging from 50 mg/l to 500 mg/l in water. To 2.85 ml FRAP reagent in a test tube, 150 µl mango pulp extract (0.1 mg/ml, in methanol) or standard was added. The mixture was incubated for 30 min in the dark, and its absorbance was measured at 593 nm. The blank contained an equal volume of methanol instead of the mango sample. The results were reported as µg of ascorbic acid equivalents (AAE) per ml.

2.5 Statistical Analysis

Three factorial experiment (maturity stage, ethylene treatment and storage period) was conducted and analysis of variance table was used to conduct a mixed factorial statistical analysis. All the analysis was performed in triplicates and the results are presented as mean ± standard deviation. Differences between variables were tested for significance by two-way analysis of variance (ANOVA) using (SAS version 9.1, Statistical Analysis System Institute, Inc. Cary, NC).

Table 1: Antioxidant content of mangoes ripened by three ripening processes during 4th, 8thand 12th day of storage**.**

	Maturity stage					
	7-9°brix			9-11°brix		
Treatment/Day of storage	**Control**	**100ppm**	**150ppm**	**Control**	**100ppm**	**150ppm**
Vitamin C (mg/100g)						
4th day	19.09±0.00^{g}	21.09±1.03^{h}	19.54±0.64^{g}	25.36±0.13^{i}	21.50±0.45^{h}	19.22±0.19^{g}
8th day	8.78±0.06^{d}	7.14±0.00^{c}	6.37±0.18^{b}	8.92±0.00^{d}	10.52±0.25^{e}	15.32±0.23^{f}
12th day	2.80±0.08^{a}	2.80±0.08^{a}	2.86±0.01^{a}	2.18±0.06^{a}	2.79±0.10^{a}	2.75±0.15^{a}
S E VALUE	0.70					
Total Carotene(µg/100g)						
4th day	48.40±0.57^{a}	93.64±0.06^{c}	91.50±0.42^{b}	48.40±0.57^{a}	93.64±0.06^{c}	91.50±0.42^{b}
8th day	226.62±0.25^{d}	285.59±0.47^{h}	257.06±0.03^{e}	273.76±0.00^{f}	273.52±0.34^{f}	276.06±0.03^{e}
12th day	369.36±0.00^{i}	617.14±0.88^{m}	697.88±1.41^{n}	587.00±1.41^{l}	456.40±0.56^{k}	448.90±0.54^{j}
S E VALUE	0.35					
Total Phenols (mg GAE/100g)						
4th day	210.98±1.26^{n}	211.63±0.34^{n}	97.84±0.71^{a}	111.93±1.16^{c}	194.11±0.24^{l}	175.55±1.20^{i}
8th day	187.24±1.73^{k}	184.46±1.56^{j}	172.88±0.96^{h}	169.78±1.37^{g}	95.92±1.47^{a}	109.04±0.52^{b}
12th day	177.73±1.36^{i}	167.35±1.31^{f}	204.97±0.25^{m}	162.53±1.20^{e}	148.56±0.67^{d}	146.98±1.30^{d}
S E VALUE	2.35					
Total Flavonoids (mg QE/100g)						
4th day	5.93±0.71^{a}	7.60±0.15^{b}	7.46±0.15^{ab}	12.89±0.25^{f}	11.17±0.56^{de}	8.10±0.25^{b}
8th day	11.92±0.81^{ef}	8.64±0.10^{bc}	11.39±0.25^{ef}	9.71±0.40^{cd}	29.25±2.06^{k}	21.82±1.16^{j}
12th day	22.50±0.71^{i}	15.21±0.51^{g}	18.46±0.96^{i}	16.39±0.06^{gh}	17.46±0.35^{hi}	11.71±0.00^{ef}
S EVALUE	1.56					

Note: All the values are expressed as mean ± SD. Values with similar superscripts within rows and columns are statistically similar at 0.05% level.

Table 2: Antioxidant content of mangoes ripened by three ripening processes during 4th, 8th and 12th day of storage.

	Maturity stage					
	7-9°brix			9-11°brix		
Treatment/Day of storage	**Control**	**100ppm**	**150ppm**	**Control**	**100ppm**	**150ppm**
Total Antioxidant Activity (mg AAE/ml)						
4th day	36.00±1.41^{d}	65.50±0.71^{l}	41.50±0.71^{e}	34.00±0.00^{cd}	42.00±1.41^{e}	28.50±0.71^{a}
8th day	62.00±1.41^{k}	44.50±0.71^{fg}	47.50±0.71^{h}	35.50±0.71^{cd}	33.50±0.71^{bcd}	31.50±0.71^{b}
12th day	27.50±0.71^{a}	56.50±0.71^{j}	52.50±0.71^{i}	34.50±0.71^{cd}	43.00±2.83^{ef}	46.00±0.00^{gh}
S E VALUE	2.18					
Reducing Power (%)						
4th day	422.13±0.52^{o}	316.50±0.00^{k}	375.50±0.71^{o}	207.50±2.12^{a}	269.75±1.06^{g}	311.52±0.67^{j}
8th day	324.50±0.71^{l}	253.75±0.35^{e}	267.01±0.69^{f}	312.97±0.74^{j}	235.12±0.53^{c}	360.89±0.15^{n}
12th day	271.92±0.82^{h}	280.76±0.36^{i}	234.41±0.48^{c}	350.94±1.34^{m}	228.83±1.18^{b}	251.00±1.41^{d}
S E VALUE	1.89					
Superoxide Anion radical Scavenging Activity (%)						
4th day	88.00±0.00^{j}	90.75±1.06^{k}	90.25±0.35^{k}	73.30±0.42^{d}	78.20±0.42^{fg}	70.25±0.35^{c}
8th day	68.25±0.35^{b}	70.00±0.71^{c}	66.75±1.06^{a}	82.35±0.49^{h}	82.75±0.35^{h}	84.75±1.06^{i}
12th day	74.75±1.06^{e}	68.50±0.00^{b}	67.75±1.06^{ab}	79.00±0.71^{g}	78.75±0.35^{g}	77.25±0.35^{f}
S E VALUE	1.44					
FRAP (µg AAE/ml)						
4th day	40.77±1.41^{b}	52.80±1.06^{e}	47.67±0.92^{d}	37.54±0.53^{a}	45.24±0.25^{c}	49.30±0.32^{d}
8th day	55.92±1.06^{g}	73.67±0.00^{k}	54.23±0.97^{ef}	64.54±1.94^{i}	66.67±0.00^{j}	55.48±0.97^{fg}
12th day	67.67±1.41^{j}	58.67±0.88^{h}	49.02±0.35^{d}	63.67±0.35^{i}	58.42±0.00^{h}	54.54±0.88^{fg}
S E VALUE	1.66					
H_20_2 Scavenging Activity (%)						
4th day	13.62±0.23^{b}	21.00±0.69^{ef}	19.10±0.71^{cd}	18.13±0.24^{c}	20.48±0.84^{de}	18.54±0.87^{c}
8th day	22.21±0.29^{fg}	20.97±1.27^{ef}	13.68±1.45^{b}	19.45±0.73^{cde}	12.84±0.17^{ab}	22.20±0.14^{fg}
12th day	37.63±1.36^{j}	28.21±0.28^{h}	18.47±0.90^{c}	34.37±0.42^{i}	23.59±0.61^{g}	11.81±0.15^{a}
S E VALUE	1.60					
DPPH (%)						
4th day	68.48±1.48^{g}	74.53±0.93^{h}	80.88±1.38^{j}	78.60±0.32^{i}	68.66±0.35^{g}	61.89±1.27^{f}
8th day	61.15±1.10^{f}	76.66±1.10^{i}	41.62±1.65^{de}	34.36±0.14^{b}	29.82±0.20^{a}	38.54±0.47^{c}
12th day	86.41±0.20^{k}	86.13±0.19^{k}	82.59±1.48^{j}	43.04±0.96^{e}	40.69±0.98^{d}	40.19±0.17^{cd}
S E VALUE	2.07					

Note: All the values are expressed as mean ± SD. Values with similar superscripts within rows and columns are statistically similar at 0.05% level

3. Results and Discussion

3.1 Results of vitamin C

Vitamin C, also known as ascorbic acid (AA), is considered as an enzymatic cofactor. It plays a key role as an essential compound for plant tissues due to its considerable antioxidant role [31]. Vitamin C is one of the most potent antioxidant vitamins required for growth, healthy body tissue and wound repair and for an efficient immune system. Vitamin C content decreased for all the treatments in both the maturity stages and was statistically similar on 12th day for all the treatments. Highest vitamin C content was exhibited by 4th day control mangoes harvested at 9-11°brix TSS followed by 4th day 100ppm ethylene treated mangoes harvested in both maturity stages. A study done on 4 mango varieties reported that vitamin C content decreases as the storage period of mangoes increases by Azad *et al* [32]. Similarly Appiah *et al*. [33] observed that vitamin C content in Keitt mango decreased during different stages of ripening. Decrease in vitamin C is attributed to susceptibility of Vitamin C to oxidative destruction during ripening [34]. The reduction in vitamin C content of the fruit during ripening may be due to the susceptibility of ascorbic acid to oxidative destruction particularly at high ambient storage temperature [35].

3.2 Results of Total carotene

Total carotene content was significantly higher for 12th day 150ppm ethylene (697.88±1.41 µg/100g) treated mango followed by 100ppm ethylene (617.14 ± 0.88 µg/100g) treated mangoes harvested at 7-9°brix TSS and control mangoes (587.00 ± 1.41 µg/100g) harvested at 9-11°brix TSS. There was an increase in the carotene content during the storage from 4th day to 12th day in all the mangoes harvested at 7-9°brix and 9-11°. The increase in carotene content was higher for artificially ripened (100ppm and 150ppm) mangoes harvested at 7-9°brix TSS as compared to control mangoes on 12th day, whereas in 9-11°brix harvested mangoes, the increase was more for control mangoes in comparison to artificially ripened mangoes. The results indicate that higher dose of ethylene treatment at 150ppm, lead to degradation of chrolophyll and formation of carotenoid compounds much faster than treatment with 100ppm ethylene. Similar results were found by Khawas *et al.* [36], Ellong *et al.* [37] in banana and mango respectively. However the increase in carotenoid content in control mangoes harvested at 9 - 11°brix was much higher than the mangoes treated with ethylene, which also indicates that mangoes harvested at physiological maturity will not need any artificial ripening, as they ripen naturally on their own.

3.3 Results of total phenols

Highets total phenol content was was found in 4th day control mangoes followed by 12th day in 150ppm ethylene treated 7-9°brix harvested mangoes. 8th day samples of all the treatments in commercial maturity stage and 4th day 100ppm ethylene treated physioloigically matured mangoes also had good phenol content. Total phenol content reduced during storage in control and 100ppm ethylene treated mangoes harvested at 7-9°brix, whereas increasein phenols content during storage was seen in 150ppm ethylene treated mangoes. Laura *et al.* [38] reported that total phenolic content in papaya in different ripening stages decreases during ripening with the highest values recorded for ripening stage 1. Miletic *et al.* [39] reported that total phenol content in fruits steadily decreases and then increases during the ripening process. Similarly Hdider *et al.* [40] reported that phenolics content in Donald, HLY 02 and Kalvert cultivar of tomato increased and then decreased during ripening whereas in Lyco 2 HLY 18, phenol content decreased and then increased during ripening. The response of Folin-Ciocalteu reagent depends on chemical structures of phenolics which are of various types and of different polarities.

3.4 Results of Total flavonoid

Flavonoids also are most promising polyphenolic compounds [41], which exhibit antioxidant activity. Total flavonoid content was found to be highest in 8th day 100ppm ethylene (29.25 ± 2.06 mg QE/100g) treated mangoes harvested at 9-11°brix TSS (physiological maturity) followed by 12th day control (22.50 ± 0.71 mg QE/100g) mangoes harvested at 7-9°brix TSS. In 7-9°brix TSS harvested mangoes, the 12th day samples in all the treatments had high total flavonoid content when compared to all other days of storage, whereas in 9-11°brix TSS harvest control mangoes had highest flavonoid content on 12th day of storage. Artificially ripened (100ppm and 150ppm) mangoes on 8th day of storage had the highest flavonoid content. Variation in flavonol content in fruits is strongly influenced by extrinsic factors such as fruit type and growth, season, climate, degree of ripeness, food preparation and processing [42, 43, 44]. A study done by Zuhair *et al.* [41] reported that total flavonoid content increases significantly during the ripening of papaya through different maturity stages from RS1 to RS5, whereas Mahmood *et al*. [45] reported that korrona and tufts species of strawberry showed an increase in flavonoid content during maturation of fruit, whereas Mulberry macroura showed an increase and then a decrease during the maturation of fruit. Similarly Hdidier *et al*. [40] observed that flavonoid content in Donald, Lyco 2, HLY 13, HLY 18 and Kalvert cultivar in tomato increased and later decreased during the ripening process.

3.5 Results of Total antioxidant activity

The antioxidant activity exhibited in fruits is mainly due to the phenolic compounds, which have redox properties, and can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [46]. Total antioxidant activity was determined in terms of mg AAE/ml of the ethanolic mango pulp extract. Results indicated an increasing trend in the antioxidant activity with the increasing days of storage with an exception of 100ppm 7-9°brix and 9-11°brix TSS harvested mangoes. The highest activity was exhibited by 7-9°brix TSS 4th day 100ppm ethylene treated mangoes followed by 8th day control mangoes. 150ppm ethylene treated mangoes antioxidant activity was not at par with the control and 100ppm ethylene treated mangoes.

3.6 Results of reducing power

Reducing power was reported to be highest for 4th day naturally ripened mangoes followed by artificially ripened mangoes (100ppm and 150ppm) harvested at commercial maturity. However control mangoes on 8th day for both maturity stage and on 12th day for 9-11°brix had a good Reducing power thus leading to a conclusion that naturally ripened mangoes in both the maturity stages during the storage will exhibit good reducing power. However 9-11°brix harvested 8th day artificially ripened mangoes (100ppm and 150ppm) also had

good reducing power indicatingthat ripening the manjira mangoes harvested at physioloigical maturity will also have high reducing power in mangoes during storage. During the storage period it was observed that the reducing power decreased from 8th day to 12th day of storage which may be due to over ripening of mangoes especially in case of 150ppm ethylene treated mangoes which became very soft on the 12th day of storage. A study done by Gull *et al.* [47] reported a significant decrease ($p>0.05$) in the reducing potential of guava fruit extract made from fruit collected from three different localities at 2.5, 5.0, 7.5 and 10.0mg/L concentration of extract during the ripening process.

3.7 Results of DPPH activity

The DPPH is decolorized by accepting an electron donated by an antioxidant. The reducing potential of a substrate usually depends on the concentration of reductants [48], which exhibit antioxidant activity by donating a hydrogen atom and breaking the chain of free radicals. The increase in concentration of antioxidants is linked to increasing the scavenging of DPPH and thus an indication of higher antioxidant activity [49]. DPPH activity was highest for 12th day control and 100ppm ethylene treated followed by same day 150ppm ethylene treated commercial harvest. However for 9-11°brix harvest, 4th day mango samples exhibited good DPPH activity in control mangoes followed by 100ppm and then 150ppm ethylene treated mangoes. Control mangoes during storage had a decrease and then an increase in DPPH activity which was same for artificially ripened mangoes (100ppm and 150ppm), with the exception of 100ppm ethylene treated 7-9°brix harvest where DPPH activity increased during the storage period from 4th day to 12th day. Sangudom *et al.* [50] and Gull *et al.* [47] reported an increase in DPPH activity in banana and guava respectively whereas, Iloki *et al.* [51] reported that DPPH activity in *Morinda citrifolia* L. (noni) harvested during month of February through March decreased and then increased significantly during ripening from stage 1 to 2 and to stage 3 whereas for fruits harvested at the months from May through June. DDPH activity remained fairly constant between ripeness stages. The only significant difference was a significant decrease detected in the stage 3 which leads to the conclusion that antioxidant activity can vary considerably with the changes in environmental conditions within the same maturity stage.

3.8 Results of Superoxide radical scavenging activity

Superoxide anion radical scavenging activity was highest on 4th day in 7-9°brix TSS harvested artificially ripened mangoes [100ppm (90.75±1.06) and 150ppm (90.25±0.35)]. The 8th day mangoes harvested at physiological maturity also exhibited good scavenging activity. The activity also decreased and the increased during the storage in 7-9°brix harvested mangoes, whereas in 9-11°brix harvested mangoes, there was an increase and then decrease during the storage period of mangoes. These results indicate that manjira mangoes consumption on 4th day for commercial maturity harvest and on 8th day for physiological harvest will exhibit higher superoxide anion radical scavenging activity by scavenging harmful ROS and free radicals such as hydrogen peroxide and hydroxyl radical from the human body which can induce oxidative damage [52].

3.9 Results of H_2O_2 scavenging activity

Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups [53, 54]. Results of estimation of H_2O_2 scavenging activity of mango pulp revealed that control mangoes without any ripening treatment on the 12th day of storage had the highest H_2O_2 scavenging activity followed by 12th day 100ppm ethylene treated mangoes in both commercial and physiological maturity stages. Thus naturally ripened manjira mangoes without any artificial ripening treatments have the potential of exhibiting good H_2O_2 scavenging activity in the body. Hydrogen peroxide is a weak oxidizing agent which can cross biological membranes and produce hydroxyl radicals leading to cytotoxicity. Thus removing H_2O_2 is very important for the protection of living systems [55], and the results of study shows that naturally ripened mangoes if consumed on 12th day, will provide with highest protection in living systems through H_2O_2 scavenging activity.

3.10 Results of FRAP activity

The FRAP activity was determined in terms of ascorbic acid equivalents. The results of the assay revealed that 100ppm ethylene treated mangoes harvested at 7-9°brix (73.67±0.00) as well as 9-11°brix (66.67±0.00) on day 8 of storage had the highest FRAP activity followed by control mangoes on day 12 of storage in both the maturity stages. 150ppm ethylene treated mangoes also exhibited the FRAP activity, but it was significantly less than control and 100ppm ethylene treated mangoes. Denies *et al.*[56]., Moneruzzaman *et al.*[57] and Zuhair *et al.*[41], reported that FRAP increases during maturation and ripening in Ivorian Gnagnan berries, pineapple and papaya fruit respectively. However Ding and Syazwani,[58] reported that FRAP activity in MD-2 pineapple fruit extract increased significantly to RS3 stage and then decreased significantly till RS5 maturity stage in both methanolic and water extract. Control mangoes had a significantly increasing trend for FRAP activity during the storage whereas artificially treated mangoes showed an increase and then decrease in the FRAP activity, thus consuming control mangoes on 12th day and artificially ripened mangoes (100ppm) on 8th day of storage day will be beneficial to the health..

4. Conclusion

Naturally ripened manjira mangoes exhibited good phenol content, reducing power, H_2O_2 scavenging activity and DPPH activity whereas artificially ripened mangoes had good carotene content, flavonoid content, total antioxidant activity, superoxide radical scavenging activity and FRAP activity. In conclusion, manjira mangoes without any artificial ripening will contribute to good antioxidant activity. Ethylene treatment of 100ppm was also found to have a better impact on the antioxidant potential of the mangoes, whereas 150ppm ethylene treatment was found to have a negative impact on the antioxidant activity of mangoes.

5. References

1. FAO (Food and Agriculture Organization of the United States). Mango, post-Harvest operations. Fao.org. AGSI/FAO, 2002.

2. FAOSTAT (Food and Agriculture Organization of the United Nations. Statistical). Production/crops, 2012. http://faostat3.fao.org/browse/Q/QC/E.
3. Prasanna V, Prabha T, Tharanathan R. Fruit ripening phenomena–an overview, Critical Reviews Food Science Nutrition. 2007; 47:1-19.
4. Jiang Y, Joyce DC, Macnish AJ. Responses of banana fruit to treatment with 1-methylcyclopropene. Plant Growth Regulation. 1999; 28:77-82.
5. Lemmens L, Tchuenche ES, Loey AMV, Hendrickx ME. Beta-carotene isomerization in mango puree as influenced by thermal processing and high-pressure homogenization, European Food Research Technology. 2013; 236:155-163.
6. Pott I, Marx M, Neidhart S, Muhlbauer W, Carle R. Quantitative determination of β-carotene stereoisomers in fresh, dried and solar-dried mangoes (Mangifera indica L.). Journal of Agricultural and Food Chemistry. 2003; 51: 4527-4531.
7. Rincon A, Kerr WL. Influence of osmotic dehydration, ripeness and frozen storage on physicochemical properties of mango. Journal of Food Processing and Preservation. 2010; 34:887-903.
8. Sogi DS, Siddiq M, Roidoung S, Dolan KD. Total phenolics, carotenoids, ascorbic acid, and antioxidant properties of fresh-cut mango (Mangifera indica L., cv. Tommy Atkin) as affected by infrared heat treatment. Journal of Food Science. 2012; 77(11):1197-1202.
9. Stahl W, Sies H. Antioxidant activity of carotenoids. Molecular Aspects of Medicine. 2003; 24:345-351.
10. Schieber A, Ullrich W, Carle R. Characterization of polyphenols in mango puree concentrate by HPLC with diode array and mass spectrometric detection. Innovative Food Science & Emerging Technologies. 2000; 1(2):161-166.
11. Barreto JC, Trevisan MT, Hull WE, Erben G, De Brito ES, Pfundstein B, Owen RW. Characterization and quantitation of polyphenolic compounds in bark, kernel, leaves, and peel of mango (Mangifera indica L.). Journal of Agricultural and Food Chemistry. 2008; 56(14):5599-5610.
12. Erdelyi K, Kiss A, Bakondi E, Bai P, Szabo C, Gergely P, Erdodi F, Virag L. Gallotannin inhibits the expression of chemokines and inflammatory cytokines in A549 cells. Molecular pharmacology. 2005; 68(3):895-904.
13. Ribeiro S, Barbosa L, Queiroz J, Knodler M, Schieber A. Phenolic compounds and antioxidant capacity of Brazilian mango (Mangifera indica L.) varieties. Food Chemistry. 2008; 110(3):620-626.
14. Gokturk Baydar N, Ozkan G, Yasar S. Evaluation of the antiradical and antioxidant potential of grape extracts. Food Control. 2007; 18(9):1131-1136.
15. Manach C, Mazur A, Scalbert A. Polyphenols and prevention of cardiovascular diseases. Current Opinion in Lipidiolgy. 2005; 16:77-84.
16. Khattak K, Simpson T, Ihasunullah A. Effect of gamma irradiation on the extraction yield, total phenolic content and free radical-scavenging activity of Nigella Sativa seed. Food Chemistry. 2008; 110:967-972.
17. Ioannou I, Ghoul M. Biological Activities and Effects of Food Processing on Flavonoids as Phenolic Antioxidants. In Petre, Marian (ed.) Advances in Applied Biotechnology, 2012. ISBN: 978-953-307-820-5.
18. Kavitha C, Kuna Aparna, Supraja T, Sagar S Blessy, Padmavathi TVN, Prabhakar Neeraja. Effect of gamma irradiation on antioxidant properties of ber (Zizyphus mauritiana) fruit. Journal of Food Science and Technology. 2015; 52(5):3123–3128. doi: 10.1007/s13197-014-1359-x.
19. Sindhu ER, Preethi KC, Kuttan R. Antioxidant activity of carotenoid lutein in vitro and in vivo. Indian Journal of Experimental Biology. 2010; 48:843-848.
20. Rajamanikandan S, Sindhu T, Durgapriya D, Sophia D, Ragavendran P, Gopalakrishnan VK. Radical scavenging and antioxidant activity of ethanolic extract of Mollugo nudicaulis by in vitro assays. Indian Journal of Pharmaceutical Education and Research. 2011; 45:310-316.
21. Ngereza Andrew Jacob, Pawelzik Elke. Antioxidant Capacity in Organically and Conventionally Grown Mango (Magnifera indica L.) and Pineapple (Ananas cosmos) Academia Journal of Agricultural Research. 2016; 4(2):053-062. doi: 10.15413/ajar.2016.0211
22. Scalfi L, Fogliano V, Pentagelo A, Graziani G, Giordano I, Ritieni A. Antioxidant activity and general fruit characteristics in different ecotypes of Corbarini small tomatoes. Journal of Agricultural and Food Chemistry. 2000; 48:1363-1366.
23. Zakaria H, Sinpson K, Brown PR, Krotatin A. Use of reversed phase high performance liquid chromatographic analysis for the determination pro vitamin A in tomatoes. Journal of Chromatography. 1979; 176:109-117.
24. Lin JY, Tang CY. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effect on mouse splenocyte proliferation. Food Chemistry. 2007; 101:140-147.
25. Damiki L, Alakh NS. Antioxidant activities of three Indian commercially available Nagakesar: An in vitro study. Journal of Chemical and Pharmaceutical Research. 2011; 3(1):277-283.
26. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989; 10:1003-1008.
27. Nishimiki M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine metho-sulfate and molecular oxygen. Biochemistry and Biophysial research communications. 1972; 46:849-853.
28. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Japanese Journal of Nutrition. 1986; 44:307-315.
29. Dorman HJD, Bachmayer O, Kosar M, Hiltunen R. Antioxidant properties of aqueous extracts from selected Lamiaceae species grown in Turkey. Journal of the Agricultural and Food Chemistry. 2004; 52:762-770.
30. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power the FRAP assay. Analytical Biochemistry. 1996; 239:70-76.
31. Gomez MLPA, Lajolo FM. Ascorbic acid metabolism in fruits: Activity of enzymes involved in synthesis and degradation during ripening in mango and guava. Journal of the Science of Food and Agriculture. 2008; 88:756-762.

32. Azad M Ittafaqul, Mortuza M Golam, Al-Amin M, Naher MNA, Alam SM Khorshed. Qualitative Analysis of Mango (Mangifera indica L.) Fruits at Different Maturity Stages. The Agriculturists. 2009; 7(1&2):1-5.
33. Appiah F, Kumah P, IdunI.Effect of ripening stage on composition, sensory qualities and acceptability of Keitt mango (mangifera indica L.) chips. African Journal of Food, Agriculture, Nutrition and Development. 2011; 11(5):5096-5109. doi: 10.4314/ajfand.v11i5.70439.
34. Aina JO. Physico-chemical changes in African Mango (Irvingia gabonensis) during normal storage ripening. Food Chemistry. 1990; 36:205-212.
35. Thomas P, Oke MS. Vitamin C content and distribution in mangoes during ripening. Journal of Food Technology. 1980; 15:669-672.
36. Khawas P, Das AJ, Sit N, Badwaik LS, Deka SC. Nutritional Composition of Culinary Musa ABB at different stages of development. American Journal of Food Science and Technology. 2014; 2(3):80-87. Doi: 10.12691/ajfst-2-3-1.
37. Ellong EN, Adenet Sandra, Rochefort Katia. Physicochemical, Nutritional, Organoleptic Characteristics and Food Applications of Four Mango (Mangifera indica) Varieties. Food and Nutrition Sciences. 2015; 6:242-253.
38. Laura E Gayosso-Garcia Sancho, Elhadi M Yahia, Miguel Angel Martinez-Tellez,Gustavo Adolfo Gonzalez-Aguilar. Effect of Maturity Stage of Papaya Maradol on Physiological and Biochemical Parameters, American Journal of Agricultural and Biological Sciences. 2010; 5(2):194-203.
39. Miletic N, Popovic B, Mitrovic O, Kandic M. Phenolic content and antioxidant capacity of fruits of plum cv. 'Stanley' (Prunus domestica L.) as influenced by maturity stage and on-tree ripening. Australian Journal of Crop Science. 2012; 6(4):681-687.
40. Hdider Chafik, Ilahy Riadh, Tlili Imen, Lenucci Marcello Salvatore, Dalessandro Giuseppe. Effect of the stage of maturity on the Antioxidant Content and Antioxidant Activity of high pigment tomato cultivars grown in Italy. Food. 2013; 7(1):1-7.
41. Zuhair AAM, Sahilah, Aminah A. Influence of Ripening Stages on Antioxidant Properties of Papaya Fruit (Carica papaya L.). AIP Conference Proceedings. 2013; 1571: 696-701.
42. Lakenbrink C, Lapczynski S, Maiwald B, Engelhardt UH. Flavonoids and other polyphenols in consumer brews of tea and other caffeinated beverages. Journal of Agricultural and Food Chemistry. 2000; 48:2848-2852.
43. Trichopoulou A, Vasilpoulou E, Hollman P, Chamalides Ch, Foufa E, Kaloudis Tr, Kromhout D, *et al*. Nutritional composition and flavonoid content of edible wild greens and green pies: a potential rich source ofantioxidant nutrients in the Mediterranean diet. Food Chemistry. 2000; 70:319.
44. Vuorinen H, Maata K, Torronen R. Content of the flavonols myricetin, quercetin, and kaempferol in Finnish berry wines. Journal of Agricultural and Food Chemistry. 2000; 48:2675-2680.
45. Mahmood Tahir, Anwar Farooq, Abbas Mateen, Saari Nazamid. Effect of Maturity on Phenolics (Phenolic Acids and Flavonoids) Profile of Strawberry Cultivars and Mulberry species from Pakistan. International Journal of Molecular Sciences. 2012; 13:4591-4607. doi: 10.3390/ijms13044591.
46. Mishra SL, Sinhamahapatra PK, Nayak A, Das R, Sannigrahi S. In vitro antioxidant potential of different parts of Oxoxylum indicum: A comparative study. Indian Journal Pharmaceutical Sciences. 2010; 72:267-269.
47. Gull Javaria, Sultana Bushra, Anwar Farooq, Naseer Rehana, Ashraf Muhammad, Ashrafuzzaman M. Variation in Antioxidant Attributes at Three Ripening Stages of Guava (Psidium guajava L.) Fruit from Different Geographical Regions of Pakistan, Molecules. 2012; 17: 3165-3180.
48. Duh PD, Tu YY, Yen GC. Antioxidant activity of water extract of Harng Jyur (Chrysanthemum morifolium Ramat). Lebensmittel-Wissenchaft & Technologie. 1999; 32:269-277.
49. Gordon MH. The Mechansism of the Antioxidant Action in Vitro. In Hudson, B.J.F (ed.) Food Antioxidants, Elsevier, London, UK. 1999, 1-18.
50. Sangudom T, Wongs-Aree C, Srilaong V, Kanlayanarat S, Wasusri T, Noichinda S, Markumlai W. Fruit quality and antioxidant properties of 'KluaiKhai' banana (Musa AA group) at different stages of harvest maturity. International Food Research Journal. 2014; 21(2):583-588.
51. Iloki Assanga SB, Lewis Lujan LM, Rivera-Castaneda EG, Gil- Salido AA, Acosta-Silva AL, Meza-Cueto CY, Rubio-Pino JL. Effect of maturity and harvest season on antioxidant activity, phenolic compounds and ascorbic acid of Morinda citrifolia L. (noni) grown in Mexico with track change). African Journal of Biotechnology. 2013; 12(29):4630-4639.
52. Al-Mamun M, Yamaki K, Masumizu T, Nakai Y, Saito K, Sano H, Tamura Y. Superoxide anion radical scavenging activities of herbs and pastures in Northern Japan determined using electron spin resonance spectrometry. International Journal of Biological Science. 2007; 3:349-355.
53. Kumaran A, Karunakaran RJ. Invitro antioxidant activities of methanol extract of Phyllanthus species from India. LWT- Food Science and Technology. 2007; 40:344-352.
54. Gulcin I, Oktay M, Kirecci E, Kufrevioglu OI. Screening of antioxidant and antimicrobial activities of anise (Pimpinella anisum L.) seed extracts. Food Chemistry. 2003; 83:371-382.
55. Rajamanikandan S, Sindhu T, Durgapriya D, Sophia D, Ragavendran P, Gopalakrishnan VK. Radical scavenging and antioxidant activity of ethanolic extract of Mollugo nudicaulis by in vitro assays. Indian Journal of Pharmaceutical Education and Research. 2011; 45:310-316.
56. Denis ND, Luca C, Teresa M, Francesca S, Massimiliano R, Daniele DR, Nicoletta P, Furio B. Effects of Different Maturity Stages on Antioxidant Content of Ivorian Gnagnan (Solanum indicum L.) Berries. Molecules. 2010; 15:7125-7138.
57. Moneruzzaman KM, Hossain ABMS, Sani W, Saifuddin M. Effect of stages of maturity and ripening conditions on the biochemical characteristics of tomato. American Journal of Biochemistry and Biotechnology. 2008; 4(4): 336-344.

58. Ding P, Syazwani, S. Physicochemical quality, antioxidant compounds and activity of MD-2 pineapple fruit at five ripening stages. International Food Research Journal. 2016; 23(2):549-555.