

# Bioactive components, antioxidative properties and inhibition of Fe<sup>2+</sup>-induced lipid peroxidation of mango peel as affected by the storage of mango fruit

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## Abstract

This study sought to evaluate the bioactive components (total phenolics, vitamin C and flavonoid), antioxidant properties (FRAP, and hydroxyl, DPPH and ABTS radical scavenging abilities) and inhibition of Fe<sup>2+</sup>-induced lipid peroxidation of the peel of mango fruit stored at refrigeration temperature and room temperature. The peel of mango fruit stored at room temperature had significantly ( $P \leq 0.05$ ) higher contents of total phenolic (13.61 mg GAE/g), vitamin C (12.98 mg AAE/g), total flavonoid (4.49 mg QE/g) and non-flavonoid (9.12 mg Qe/g) than the peel of freshly harvested mango fruit and the peel of mango fruit stored at refrigeration temperature. In consonance with the bioactive components, the peel of mango fruit stored at room temperature had a higher FRAP, and hydroxyl, DPPH and ABTS radical scavenging abilities than the others. The peel of mango fruit stored at room temperature showed stronger inhibition of Fe<sup>2+</sup> induced lipid peroxidation by exhibiting the least IC<sub>50</sub> (1.44 mg/ml in brain), (1.43 mg/ml in pancreas) and (1.88 mg/ml in kidney). Thus freshly harvested, matured, edible and just ripe mango fruit (Sheri Mango) could be stored at room temperature and be consumed with the peel.

**Keywords:** Mango peel; Storage; Total phenolic; Vitamin C; Flavonoid; Lipid peroxidation

## 1 Introduction

Mango is a fruit that belongs to the genus *Mangifera indica*, consisting of numerous species of tropical fruiting trees in the flowering plant family *Anacardiaceae* (Sarkiyayi, Mohammed, & Yakubu, 2013). Mango (*Mangifera indica*) is a widely popular fruit grown in tropical regions of the world because of its sweet taste and high nutritive content. The mango constituents are: pulp 33-70%, kernel 7-24% and peel 15-20% of the total fruit weight (Imran, Butt, Anjum, & Sultan, 2013). Mango is used as food in all stages of its development. Green or unripe mango contains a very high portion of starch which grad-

ually changes into glucose, sucrose and maltose as the fruit begins to ripen and disappears completely when the fruit is fully ripened (Sarkiyayi et al., 2013).

Nutritionally, mango peel contains moisture 68.50%, protein 2.05%, ash 2.62%, fibre 5.40% and carbohydrates 26.50% and has an energy content of 453.92 kJ/100g (Imran et al., 2013). Polyphenolic compounds, abundantly found in mango, not only provide various functionalities in foods but also act as a strong antioxidant in the body. Mango peel is a promising source of phytonutrients such as polyphenols, carotenoids and vitamin E and C. It is to be noted that

polyphenol content in mango peel is much higher than that of mango pulp (Ajila, Naidu, Bhat, & Rao, 2007).

In 1993 it was estimated that 1,164,320 tonnes of mango were produced in Nigeria. Nigeria occupied the eighth position in the world ranking of mango producing countries in 2002 (Oyedoyin, Akinola, Ajuebor, Omotade, & Kupoluyi, 2008; Yusuf & Salau, 2007). In Nigeria, mango processing into juice is uncommon since mango has not been cultivated on a commercial scale. When consuming mango in Nigeria, the peels are always removed and discarded, the pulp is consumed and the seeds are thrown away. Mango is seasonal and predominant in the southern part of Nigeria. During the mango season, mangoes need to be transported to other parts of the country (Urban centres) where they command better prices. Due to the poor road network from the outlying villages to the markets in Nigeria, produce normally spends four to five days in transit, out of which two days are spent on movement from the production site to the market and the remaining days spent at source markets (Adetuyi, Ibrahim, Ajalia, & Oloye, 2010).

To our knowledge, there has been no previous study to directly compare phenolics, vitamin C and antioxidant properties of mango peel, with respect to storage of mango fruit. However, phenolics and antioxidant properties of mango peel has been previously evaluated (Ajila et al., 2007; Kim, Brecht, & Talcott, 2007; Tunchaiyaphum, Eshtiaghi, & Yoswathana, 2013; De Almeida Monaco, Costa, Uliana, & Lima, 2014). The present study was designed to evaluate the bioactive components, antioxidant properties and inhibition of Fe<sup>2+</sup>-induced lipid peroxidation of mango peel as affected by storage of mango fruit at room and refrigeration temperatures.

## 2 Materials and Methods

### 2.1 Materials

The maturity stages of mango fruit used in this study were evaluated visually based on the skin colour as defined by Bron and Jacomino (2006): Stage 0 - totally green; Stage 1 - yellow colour that does not cover more than 15% of skin

surface; Stage 2 - fruit with 16-25% of yellow skin; Stage 3 - fruit with 26-50% of yellow skin. Freshly harvested, matured, just ripe mango fruit (Sheri Mango variety) at stage 1, with slight appearance of yellow colour, obtained from the Ondo State University of Science and Technology farm was used for this study. The mango fruit was harvested in March.

### 2.2 Sample preparation

The mango fruits were divided into two lots; one was stored on the bench in the laboratory at room temperature ( $28 \pm 1$  °C) and the other one was stored in a refrigerator ( $10 \pm 1$  °C) for 72 hours. The mango peel of the freshly harvested and the stored mango fruit was taken and dried in the oven at 50 °C for 8 hours. The dried samples (200 g) were milled and sieved, then packed in plastic bags and kept at 4 °C until required. All determinations were done in triplicate for Vitamin C, Total phenolic, Flavonoid, ABTS scavenging ability, ferric reducing antioxidant property (FRAP), Fenton reaction, DPPH free radical scavenging ability and lipid peroxidation.

### 2.3 Aqueous extract preparation

The aqueous extract of mango peel was prepared using a modified method described by Oboh, Ademiluyi, and Akindahunsi (2010). Two grams (2 g) of the powdered mango peel was homogenised in 100 ml distilled water in a Warring blender for 5 min. Thereafter, the mixture was centrifuged at 2000 g for 10 min. The supernatant was used for the determination of the antioxidant content and properties of mango peel.

### 2.4 Vitamin C content determination

The vitamin C content of the aqueous extract was determined using the method of Benderitter et al. (1998). 75 µl DNPH (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg CuSO<sub>4</sub>·5H<sub>2</sub>O in 100 ml of 5 ml H<sub>2</sub>SO<sub>4</sub>) were added to 500 µl extracts mixture (300 µl of an appropriate dilution of the extract with 100 µl 13.3 %

trichloroacetic acid (TCA) and water). The reaction mixture was subsequently incubated for 3 hours at 37 °C, then 0.5 ml of 65 % H<sub>2</sub>SO<sub>4</sub> (v/v) was added to the medium and the absorbance was measured at 520 nm in a JENWAY UV-Visible spectrophotometer (JENWAY 6305 Barloworld Scientific Ltd. Dunmow, Essex, UK). The vitamin C content of the extracts was subsequently calculated using ascorbic acid as standard.

## 2.5 Total Phenolic content determination

The total phenol content was determined according to the method of Singleton, Orthofer, and Lamuela-Raventos (1999). Appropriate dilutions of the extracts were mixed with 2.5 ml of 10 % Folin-Ciocalteu's reagent (v / v) and neutralised by 2.0 ml of 7.5 % sodium carbonate. The reaction mixture was incubated for 40 min at 45 °C and the absorbance was measured at 765 nm in a spectrophotometer (JENWAY 6305). The total phenol content was subsequently calculated using gallic acid as a standard.

## 2.6 Total flavonoid content determination

The total flavonoid content of the extracts was determined using a slightly modified method reported by (Meda, Lamien, Romito, Millogo, & Nacoulma, 2005). Briefly, 0.5 ml of appropriately diluted sample was mixed with 0.5 ml methanol, 50 ml of 10 % Aluminium chloride (AlCl<sub>3</sub>), 50 ml of 1 mol/L potassium acetate and 1.4 ml water, and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was subsequently measured at 415 nm using a spectrophotometer (JENWAY 6305). The total flavonoid was calculated using quercetin as a standard.

## 2.7 Ferric reducing antioxidant power (FRAP)

The reducing property of the extracts was determined by assessing the ability of the extract

to reduce FeCl<sub>3</sub> solution as described by Oyaizu (1986). A 2.5 ml aliquot was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1 % potassium ferricyanide. The mixture was incubated at 50 °C for 20 min; thereafter 2.5 ml of 10% trichloroacetic acid was added. This mixture was centrifuged at 2000 g for 10 min; 5 ml of the supernatant was mixed with an equal volume of water and 1 ml of 0.1 % ferric chloride. The absorbance was measured at 700 nm in a spectrophotometer (JENWAY 6305) and ferric reducing antioxidant property was subsequently calculated using ascorbic acid as a standard.

## 2.8 DPPH free radical scavenging ability

The Free radical scavenging ability was determined using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) as described by Singh, Murthy, and Jayaprakasha (2002). Different concentrations of the aqueous extract were taken in different test tubes and the volume was made to 1 ml with distilled water. 4 ml of 0.1 mM methanolic solution of DPPH was added. The tubes were shaken vigorously and allowed to stand for 20 min at room temperature. A control was prepared as above without the sample and distilled water was used for base line correction. Changes in absorbance of samples were measured at 517 nm in a spectrophotometer (JENWAY 6305). Free radical scavenging activity was expressed as percentage inhibition and was calculated using the following formula:

$$\text{FRAP}(\%) = \frac{(\text{control OD} - \text{sample OD})}{\text{control OD}} \times 100 \quad (1)$$

## 2.9 Degradation of deoxyribose (Fenton's reaction)

The ability of the extracts to prevent Fe<sup>2+</sup>/H<sub>2</sub>O<sub>2</sub>-induced decomposition of deoxyribose was carried out using the method of Halliwell and Gutteridge (1981). Freshly prepared aqueous extract (0–150 μl) was added to a reaction mixture containing 120 μl, 20 mM

deoxyribose, 400  $\mu\text{l}$ , 0.1 M phosphate buffer, 40  $\mu\text{l}$ , 20 mM hydrogen peroxide and 40  $\mu\text{l}$ , 500 M  $\text{FeSO}_4$ , and the volume made up to 800  $\mu\text{l}$  with distilled water. The reaction mixture was incubated at 37 °C for 30 min, and the reaction was stopped by the addition of 0.5 ml of 2.8 % trichloroacetic acid solution; this was followed by the addition of 0.4 ml of 0.6 % thiobarbituric acid solution. The tubes were subsequently incubated in boiling water for 20 min. The absorbance was measured at 532 nm in spectrophotometer (JENWAY 6305).

## 2.10 ABTS scavenging ability

The ABTS scavenging ability of the extracts were determined according to the method described by Re et al. (1999). The ABTS was generated by reacting an ABTS aqueous solution (7mM/L) with potassium persulfate ( $\text{K}_2\text{S}_2\text{O}_8$ ) (2.45 mM/L, final concentration) in the dark at room temperature for 16 hours and adjusting the Abs734 nm to 0.700 with ethanol. 0.2 ml of appropriate dilution of the extract was added to 2.0 ml ABTS solution and the absorbance was measured at 734 nm after 15 minutes in the JENWAY UV – Visible spectrophotometer. The trolox equivalent antioxidant capacity was subsequently calculated.

## 2.11 Lipid peroxidation assay

### Preparation of tissue homogenates

The rats (12 weeks old and weighing between 220 and 240 g) were decapitated under mild diethyl ether anaesthesia and the whole brain, pancreas and kidney were rapidly isolated and placed on ice and weighed. These tissues were subsequently homogenized in cold saline (1:10, w/v) with about 10 up and down strokes at approximately 1200 rpm in a Teflon glass homogenizer. The homogenate was centrifuged for 10 min at  $3000 \times g$  to yield a pellet that was discarded, and a low – speed supernatant (SI) which was kept for the Lipid peroxidation assay (Belle, Dalmolin, Fonini, Rubin, & Rocha, 2004).

## 2.12 Lipid peroxidation and TBA reactions

The lipid peroxidation assay was carried out using the modified method of Ohkawa, Ohishi, and Yagi (1979). Briefly, 100 $\mu\text{L}$  SI fraction was mixed with a reaction mixture containing 30  $\mu\text{L}$  of 0.1 M Tris-HCl buffer (pH 7.4), extract (0-100  $\mu\text{L}$ ) and 30  $\mu\text{L}$  of 250  $\mu\text{M}$  freshly prepared  $\text{FeSO}_4$ . The volume was made up to 300  $\mu\text{L}$  by water before incubation at 37 °C for 1hour. The colour reaction was developed by adding 300  $\mu\text{L}$  of 8.1 % sodium dodecyl sulphate (SDS) to the reaction mixture containing SI; this was subsequently followed by the addition of 600  $\mu\text{L}$  acetic acid/HCl (pH 3.4) mixture and 600  $\mu\text{L}$  of 0.8 % thiobarbituric acid (TBA). This mixture was incubated at 100 °C for 1 hour. Thiobarbituric acid reactive species (TBARS) produced were measured at 532 nm in the JENWAY UV-visible spectrophotometer and the absorbance was compared with that of a standard curve using malondialdehyde (MDA).

## 2.13 Statistical analysis

The results of the three replicates were pooled and expressed as mean  $\pm$  standard deviation. Standard deviations were calculated using spread sheet soft-ware (Microsoft Excel<sup>®</sup>, version 2013). Analysis of variance (ANOVA) was performed using Statistical Analysis System proprietary software (SAS, 2002). Duncan's multiple range test procedure as described in the SAS software was used for mean separations. Significance was accepted at  $P \leq 0.05$ .  $\text{IC}_{50}$  (extract concentration causing 50 % inhibition of antioxidant activity) was calculated using a linear regression analysis.

*We declared that this research work adhered strictly to the guide for the care and use of Laboratory animals according to the revised NIH publication of 1985.*

## 3 Results and Discussion

The vitamin C, total phenol, flavonoid content and ferric reducing antioxidant power (FRAP) of

Table 1: Phenolic, Ascorbic acid, total flavonoid, total non flavonoid and FRAP of the peel of stored mango (*M. indica L.*) fruit

Storage temperature	Total Phenol (mg GAE/g)	Ascorbic Acid (mg AAE/g)	Total flavonoid (mg QE/g)	Total non flavonoid (mg QE/g)	FRAP (mg AAE/g)
F. Harvested	11.90 <sup>b</sup> ±0.1	9.86 <sup>c</sup> ±0.1	3.58 <sup>b</sup> ±0.0	8.4 <sup>b</sup> ±0.1	3.56 <sup>c</sup> ±0.1
Ref. Temp.	12.24 <sup>b</sup> ±0.3	10.69 <sup>b</sup> ±0.2	3.58 <sup>b</sup> ±0.1	8.66 <sup>b</sup> ±0.1	4.44 <sup>b</sup> ±0.1
Room Temp.	13.61 <sup>a</sup> ±0.3	12.08 <sup>a</sup> ±0.2	4.49 <sup>a</sup> ±0.1	9.12 <sup>a</sup> ±0.2	5.56 <sup>a</sup> ±0.1

Values represent mean ± standard deviation of triplicate determination. Values with the same letter along the same column are not significantly different ( $P \leq 0.05$ ). GAE – Gallic Acid Equivalent, AAE – Ascorbic Acid Equivalent, QE – Quercetin Equivalent. F. Harvested = Freshly harvested mango fruit, Ref. Temp. = Mango fruit stored at refrigeration temperature, Room Temp. = Mango fruit stored at room temperature

the mango peel as affected by storage of mango fruit is presented in Table 1. It shows that the mango peel has a vitamin C content of 9.86 mg Ascorbic Acid Equivalent (AAE)/g. This was higher than the value reported by previous researchers for peeled whole mango fruit. Sarkiyayi et al. (2013) reported 34.1 mg/100g on the average for three different mango fruit in Kaduna, Nigeria. 50.60 mg/100g was reported by De Almeida Monaco et al. (2014) for the pulp of mango. Ajila et al. (2007) in the study of valuable components of raw and ripe peels from two Indian mango varieties reported vitamin C contents ranging from 188 to 392  $\mu\text{g/g}$  dry peel, with higher levels in ripe peel. The vitamin C content of the mango peel increased, slightly but significantly, in stored mango fruit. The peel of mango fruit stored at room temperature recorded 12.08 mg AAE/g which was significantly higher than the vitamin C content of freshly harvested mango and mango fruit stored at refrigeration temperature. The increase in the vitamin C content of the peel in the stored mango fruit could be as a result of ripening since the mango fruit stored at room temperature has ripened more than the freshly harvested mango fruit. Fruits generally have variable patterns during storage and ripening processes. In the course of fruit ripening, vitamin C contents may decrease, increase or remain constant (Cordenunsi, do Nascimento, Genovese, & Lajolo, 2002). Ripening has been observed to cause an increase in vitamin C content of banana and guava (Fernando, Srilaong, Pongprasert, Boonyaritthongchai, & Jitareerat, 2014; Lim, Lim, & Tee, 2006; Gull et al., 2012). It has been observed by Ortega et al. (2013) that the ascorbic acid content of an ‘Ataulfo’ mango

variety during postharvest maturation increased in storage until senescence when it starts to decrease. However, Gomez and Lajolo (2008) observed a decrease in vitamin C content of mango during ripening which is contrary to the finding of Ortega et al. (2013). The increase in vitamin C content of mango fruit with ripening may be due to physiological activities that persist after harvesting of the fruit, which is the breakdown of starch to glucose, and this causes an increase in the biosynthesis of ascorbic acid. The increase in ascorbic acid content during ripening has also been attributed to the increase in lipid peroxidation which is another physiological activity going on in the ripened fruit (Jimenez et al., 2002). The total phenolic content of the mango peel was determined and reported as mg gallic acid equivalent (GAE) per g sample as shown in Table 1. The total phenolic content of the peel of freshly harvested mango fruit was found to be 11.90 mg GAE/g. This result was similar to the result (11.68 mg GAE/g) obtained by Tun-chaiyaphum et al. (2013). The value obtained in this present study was higher than the previous values reported for different varieties of mango peels; 45.25 – 87.67 mg GAE/100g (Imran et al., 2013; Ramirez, Zambrano, Sepulveda, & Simirgiotis, 2014) and 0.86 g GAE/100g (De Almeida Monaco et al., 2014). However, this value is lower than that reported by Nixwell, Johanna, and Ngezimana (2013) at 2.4 mg/100mg for the average of six mango cultivars and Ajila et al. (2007) at 55 to 110 mg/g dry peel for two Indian mango varieties. The total phenol content of the peel of mango fruit stored at refrigeration temperature (12.24 mg GAE/g) was not significantly different from the peel of freshly harvested mango fruit.

Physiological activities continue in all plant crops following harvesting. These processes involve changes in the chemical composition and physical characteristics of the plant material and can influence its quality as food, whether it is consumed fresh or used as raw material for subsequent processing operations (Rhodes, 1980).

At low temperature enzymes and physiological activities are slowed down or stopped. This could be a cause for the non significant difference in the total phenol content of the peel of mango fruit stored at refrigeration temperature and freshly harvested mango fruit. The total phenol content of the peel of mango fruit stored at room temperature recorded the highest value (13.61 mg GAE/g) which was significantly different from the total phenol content of the peel of freshly harvested and refrigerated mango fruit. Ripening, which results in an increase in the extractability of the polyphenols can be a factor because the mango fruit at room temperature has ripened more than the freshly harvested mango fruit. Previous researchers have recorded higher total phenolic contents in ripened mango peel (Kim et al., 2007; Ajila et al., 2007; Palafox-Carlos et al., 2012). The total phenolic content of mango has been reported to be affected by level of maturity, cultivar type, agronomic practices, climatic conditions, ripeness at harvest, and the postharvest storage conditions of the fruit (Kim et al., 2007; Nixwell et al., 2013).

The flavonoid content of the peel of mango fruit as presented in Table 1 shows that the mango peel of freshly harvested mango fruit has a flavonoid content of 3.58 mg Quercetin Equivalent (QE)/g. The flavonoid content in the present study was similar to the value reported for mango peel using a polar extraction method (Toledo-Guillen, Higuera-Ciapara, Garcia-Navarrete, & de la Fuente, 2010; Garcia, Cabral, & Martinez-Correa, 2013), higher than that reported by earlier researchers for different mango peel varieties (Ramirez et al., 2014; De Almeida Monaco et al., 2014) but lower than the 11.73 QE mg/g reported by Nisha and Bhatnagar (2014). The value of the flavonoid of the peel of mango fruit stored at refrigeration temperature (3.58 mg QE/g) was not significantly different from the peel of freshly harvested mango fruit. Robles-Sanchez et al. (2009) also reported that

no changes were observed in 'Ataulfo' mangoes, stored at 5 °C and unstored for 15 days.

The flavonoid content of the peel of mango fruit stored at room temperature (4.49 mg QE/g) was significantly higher than the flavonoid content of the peel of freshly harvested mango fruit and mango fruit stored at refrigeration temperature. The increase in the flavonoid content of the peel of mango fruit stored at room temperature could be attributed to ripening. It was reported previously in a study of the antioxidant content of 'Ataulfo' mango during postharvest maturation that the concentration of flavonoids in the mango fruit reached the maximum level on day 5 and decreased gradually to reach the lowest level on day 14 (Ortega et al., 2013). The flavonoid content of fruits increased or was unchanged during ripening or storage, but during senescence, most flavonoids decreased in content (Kevers et al., 2007). Different factors, including ultraviolet rays of light, can change the metabolism of polyphenols, including flavonoids, thereby inducing their synthesis or degradation (Ortega et al., 2013). Palafox-Carlos et al. (2012) when studying the effect of ripeness stage of mango fruit on physiological parameters and antioxidant activity observed that there were no significant differences with respect to ripening stages in the flavonoid content of mango fruit.

The non-flavonoid content of the peel of freshly harvested mango fruit and stored mango fruit followed the trend observed for both the total phenol and flavonoid contents where the values for the peel of mango fruit stored at room temperature was significantly higher than others.

It is a known fact that several food components (carotenoids, vitamin C, vitamin E, phenolic compounds) and their synergy contribute to the overall antioxidant capability of the food. It is then cumbersome to assess total antioxidant capability on the basis of these individual active components (Pinelo, Manzocco, Nunez, & Nicoli, 2004). Based on this fact the antioxidants in this study were evaluated in the form of total activity. Five representative methods, Ferric reducing antioxidant power (FRAP), Hydroxyl radical scavenging ability, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability, ABTS and Lipid peroxidation and thiobarbituric acid reactions (TBARS) were selected for the measure-

ment of antioxidant activity in the mango peel as affected by storage of the mango fruit.

The reducing power of the mango peel extracts was determined based on the capacity of the extract to reduce the transition metal iron (III) by electron transfer to iron (II). A Higher absorbance at 700 nm indicated higher activity (Zarena & Sankar, 2009). The ferric reducing antioxidant power (FRAP) of the mango peel in Table 1 shows that the peel of mango fruit stored at room temperature exhibited the highest reducing power (5.56 mg AAE/g) which was significantly higher than the reducing power of the peel of freshly harvested mango fruit and mango fruit stored at refrigeration temperature. The high reducing power in the peel of mango fruit stored at room temperature is related to the high phenolic and flavonoid content of the peel which are required for reduction of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  as a result of ripening. The reducing power property indicated that the antioxidant compounds are electron donors which can reduce the oxidized intermediates of the lipid peroxidation process (Tachakittirungrod, Okonogi, & Chowwanapoonpohn, 2007). The FRAP assay is considered a good method to evaluate the antioxidant capacity of fruits, including several mango cultivars (Palafox-Carlos et al., 2012). However, the FRAP assay has a disadvantage. Any electron-donating substances with redox potential lower than that of the redox pair  $\text{Fe}^{3+}/\text{Fe}^{2+}$  can contribute to the FRAP value and indicate falsely high values even if they don't have antioxidant properties (Nilsson et al., 2005).

The hydroxyl radical ( $\text{OH}^\bullet$ ) produced from the interaction of  $\text{Fe}^{2+}$  and  $\text{H}_2\text{O}_2$  in cells through the Fenton reaction has been recognized to date as the most reactive oxygen species (ROS). The  $\text{OH}^\bullet$  can easily cross cell membranes, and attack macromolecules like DNA leading to mutations and polyunsaturated fatty acids of the cell membranes to induce lipid peroxidation (Aruoma et al., 2002; Ademiluyi & Oboh, 2011). Thus, the removal of the  $\text{OH}^\bullet$  as it is produced would prevent the oxidation of these macromolecules. Therefore, antioxidants could be evaluated through their capacity to scavenge the  $\text{OH}^\bullet$ . Figure 1 shows the hydroxyl radical scavenging ability of the peel of the freshly harvested mango fruit and stored mango fruit. The ex-

tracts of these mango peels scavenged hydroxyl radicals produced from the decomposition of deoxyribose in a dose dependent manner, with increasing concentrations from 0.36 mg/ml to 1.43 mg/ml.

The peel of the mango fruit stored at room temperature had the highest hydroxyl radical scavenging activity at the various tested concentrations. This is also reflected in the  $\text{IC}_{50}$  value in Table 2, where the peel of the mango fruit stored at room temperature recorded the lowest  $\text{IC}_{50}$  value of 1.25 mg/ml. A lower  $\text{IC}_{50}$  value indicates a stronger antioxidant activity in the sample. This is expected considering the increase in total phenol and flavonoid content of the peel of mango fruit stored at room temperature caused by ripening. An aqueous extract was used for this analysis. The total antioxidant activity of the aqueous extract cannot be predicted based on its total phenolic content alone. A synergism of soluble polyphenolic compounds, with one another, and/or other components present in the extracts, may contribute to the overall observed antioxidant activity (Moktan, Saha, & Sarkar, 2008).

The DPPH results are used to confirm the results obtained in total phenolic content (Palafox-Carlos et al., 2012). The DPPH radical-scavenging ability of the peel of freshly harvested mango and stored mango fruits at 0 – 3.33 mg/ml concentrations were measured and the results are presented in Figure 2. A dose-response relationship was found in the DPPH radical-scavenging ability of the mango peel extracts; the ability increased with an increase in the concentration of the mango peel extracts. These results show that ripening caused a significant increase in the DPPH radical-scavenging ability of mango peels. Ripening has been reported by previous researchers to cause an increase in the DPPH radical-scavenging ability of 'Keitt' mango, and 'Ataulfo' mango fruits in storage (Talcott, Moore, Lounds-Singleton, and Percival (2005) and Ortega et al. (2013)). The peel of the mango fruit stored at room temperature recorded the highest DPPH radical-scavenging ability which was significantly higher than that of the peel of freshly harvested mango fruit and mango fruit stored at refrigeration temperature. It can be seen from Table 2 that the peel of the

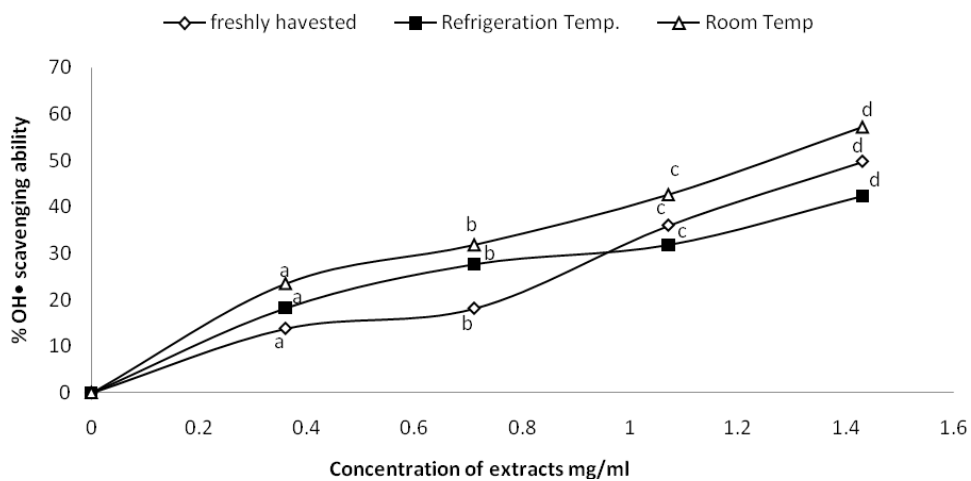


Figure 1: OH• scavenging abilities of the peel of stored mango (*M. indica L.*) fruit. Values represent mean of triplicate determination. Values with the same letter along the same column are not significantly different ( $P \leq 0.05$ )

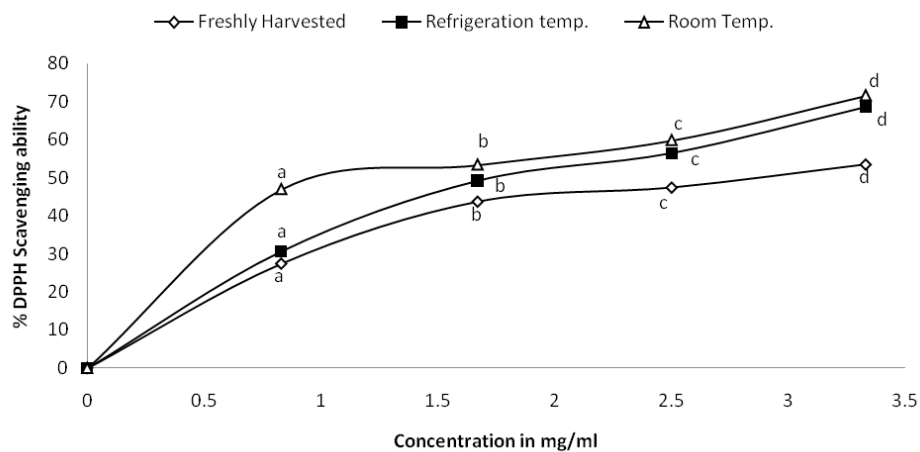


Figure 2: DPPH scavenging abilities of the peel of stored mango (*M. indica L.*) fruit. Values represent mean of triplicate determination. Values with the same letter along the same column are not significantly different ( $P \leq 0.05$ )



mango fruit stored at room temperature recorded the lowest IC<sub>50</sub> value of 1.28 mg/ml.

The ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants (scavengers of aqueous phase radicals) and of chain breaking antioxidants (scavenger of lipid peroxy radicals) (Leong & Shui, 2002). The ABTS radical has been used to confirm results obtained with the DPPH method because both possess similar antioxidant mechanisms. It has been reported that phenolic compounds or ascorbic acids react vigorously with ABTS, while lipophilic compounds make them weaker (Perez-Jimenez et al., 2008). ABTS scavenging ability, reported as Trolox Equivalent Antioxidant Capacity (TEAC), of the peel of freshly harvested mango and a stored mango fruit is presented in Figure 3. The results reveal that the ABTS scavenging ability of the peel of the mango fruit stored at room temperature (0.62 mmol. TEAC/g) was significantly higher than that of the peel of freshly harvested mango fruit (0.43 mmol. TEAC/g) and mango fruit stored at refrigeration temperature (0.52 mmol. TEAC/g). The higher the TEAC value of the sample, the stronger was the antioxidant activity (Zarena & Sankar, 2009). This trend was also observed for DPPH scavenging ability of the peel of mango fruit for both fresh and stored samples. The physiological and ripening process in mangoes directly affects the phenolic content and their antioxidant activity. In climacteric fruits like mango, the cellular activities are remarkably high during ripening when most of the biomolecules are being metabolized (Palafox-Carlos et al., 2012). During this process, the fruits produce energy by the respiratory system to support these physiological pathways in the cell and this may generate free radicals and oxygen reactive species at the end of the electron transporting chain (Bapat et al., 2010; Masibo & He, 2008). The mango fruits may need to activate antioxidants defense mechanisms in order to avoid oxidative stress, thereby activating the synthesis of phenolic compounds by the phenylpropanoid pathway.

The polyunsaturated fatty acids (PUFAs) rich phospholipids in the brain membranes are easily attacked by free radicals, causing oxidative damage to the brain phospholipids which could result

in the development of Alzheimer's disease (Axelsen, Komatsu, & Murray, 2011). Lipid peroxidation enhances the release of arachidonic acid but inhibits prostaglandin synthesis in the kidney medulla (Fujimoto, Tanioka, Keshi, & Fujita, 1983). The accumulation of Fe<sup>2+</sup>, in the acinar cells and in the islets of Langerhan, results in the oxidative destruction of the  $\beta$ -cells of the pancreas associated with Diabetic Melitus (Shah & Fonseca, 2011). The protective capacity of mango peel extracts against Fe<sup>2+</sup> induced lipid peroxidation in cultured rat brain, pancreas and kidney is shown in Figures 4, 5 and 6. Incubation of rat's brain, pancreas and kidney in the presence of 250 $\mu$ M iron (II) caused a significant increase in the MDA content of the three organs: 154.93 % in the brain, 133.33 % in the pancreas and 128.75 % in the kidney. The increase in iron in these organs resulted in the formation of reactive oxygen species (ROS) which facilitates lipid peroxidation through the Fenton reaction. This could have accounted for the increase in MDA contents of these organs after incubation in the presence of Fe<sup>2+</sup>. The results show that the aqueous extracts of the peels of freshly harvested mango fruit and stored mango fruit significantly inhibited MDA production in the brain, pancreas and kidney in a dose dependent manner. However, the peel of mango fruit stored at room temperature had the highest inhibitory effect on the Fe<sup>2+</sup> induced lipid peroxidation in rat brain, pancreas and kidney since it exhibited the least IC<sub>50</sub> (Table 2) (1.44 mg/ml for brain, 1.43 mg/ml for pancreas and 1.88 mg/ml for kidney). The possible mechanism through which the extracts protect the brain could be by Fe<sup>2+</sup> chelation and the scavenging of the OH<sup>•</sup> (Ademosun & Oboh, 2014). The ability of the extracts of the mango fruit peel, both fresh and stored mango fruit, to inhibit Fe<sup>2+</sup> induced lipid peroxidation in the pancreas homogenate is a reflection that they could prevent the oxidative damage of the pancreas cells thereby preventing the development of Diabetic Melitus.

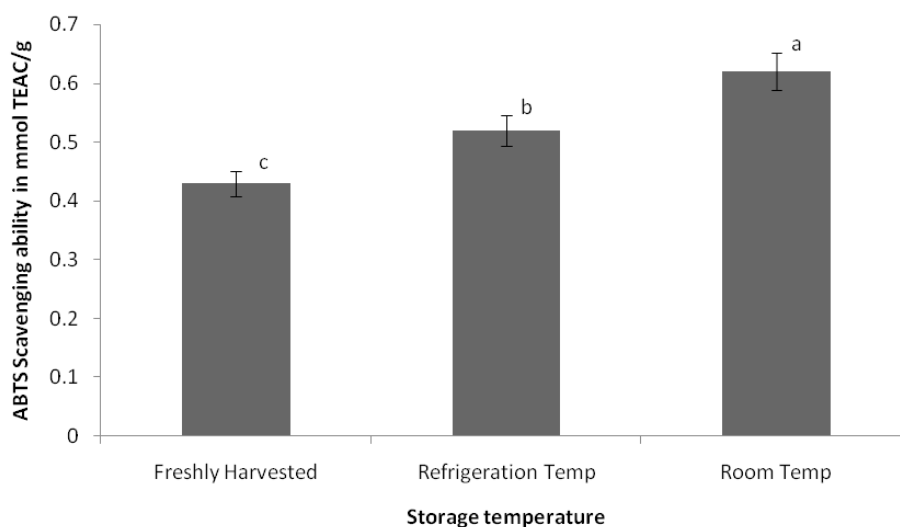


Figure 3: ABTS scavenging abilities of the peel of stored mango (*M. indica L.*) fruit. Values represent mean of triplicate determination. Values with the same letter along the same column are not significantly different ( $P \leq 0.05$ )

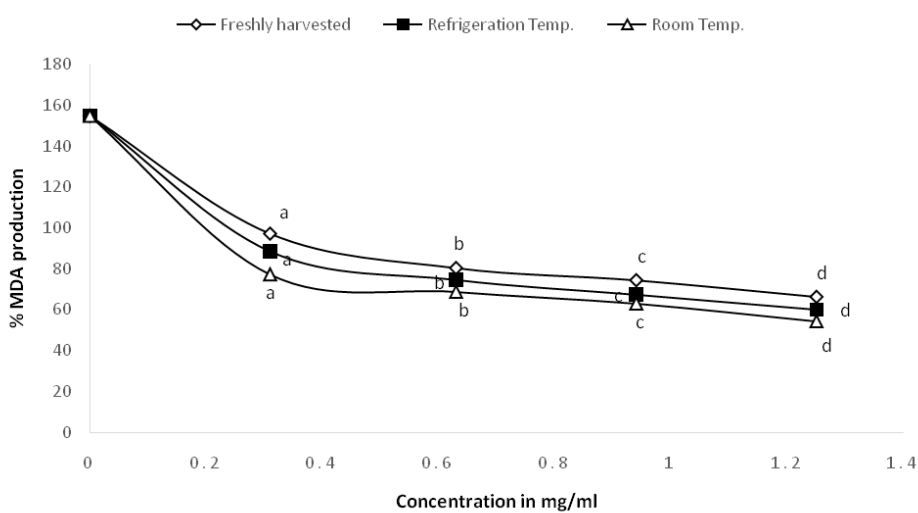


Figure 4: Inhibition of  $Fe^{2+}$  induced MDA production in rat brain by the peel of stored mango (*M. indica L.*) fruit. Values represent mean of triplicate determination. Values with the same letter along the same column are not significantly different ( $P \leq 0.05$ )

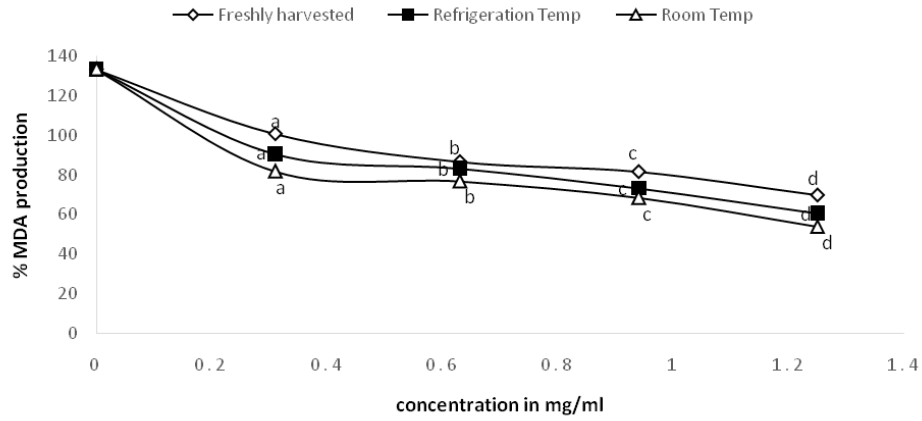


Figure 5: Inhibition of Fe<sup>2+</sup> induced MDA production in rat pancreas by the peel of stored mango (*M. indica L.*) fruit. Values represent mean of triplicate determination. Values with the same letter along the same column are not significantly different ( $P \leq 0.05$ )

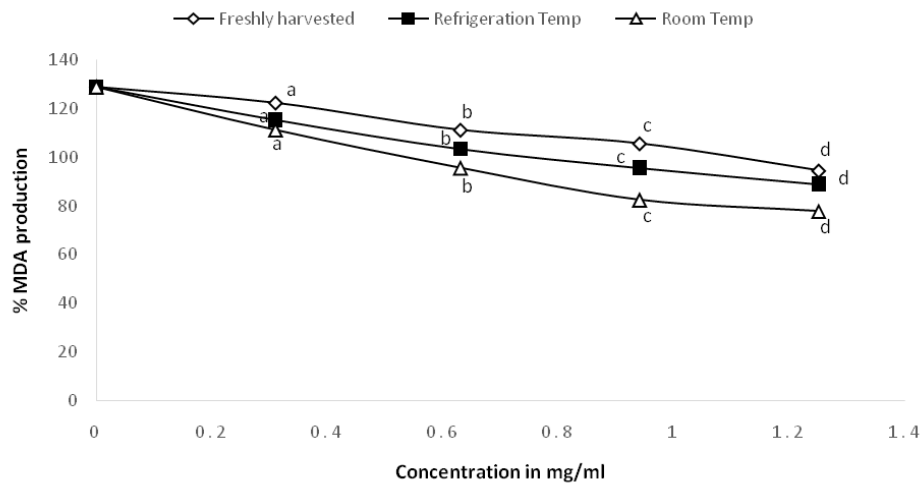


Figure 6: Inhibition of Fe<sup>2+</sup> induced MDA production in rat kidney by the peel of stored mango (*M. indica L.*) fruit. Values represent mean of triplicate determination. Values with the same letter along the same column are not significantly different ( $P \leq 0.05$ )

Table 2: IC<sub>50</sub> of OH• and DPPH scavenging abilities and inhibition of Fe<sup>2+</sup> induced MDA Production of the peel of stored mango (*M. indica L.*) fruit

Storage temperature	OH• (mg/ml)	DPPH (mg/ml)	MDA Brain (mg/ml)	MDA Pancreas (mg/ml)	MDA Kidney (mg/ml)
F. Harvested	1.45 <sup>b</sup> ±0.05	2.73 <sup>b</sup> ±0.03	1.67 <sup>b</sup> ±0.09	1.86 <sup>b</sup> ±0.05	2.81 <sup>b</sup> ±0.09
Ref. Temp.	1.80 <sup>b</sup> ±0.02	2.00 <sup>c</sup> ±0.08	1.52 <sup>c</sup> ±0.07	1.62 <sup>b</sup> ±0.09	2.57 <sup>b</sup> ±0.07
Room Temp.	1.25 <sup>a</sup> ±0.03	1.28 <sup>a</sup> ±0.07	1.44 <sup>a</sup> ±0.05	1.43 <sup>a</sup> ±0.02	1.88 <sup>a</sup> ±0.04

Values represent mean ± standard deviation of triplicate determination. Values with the same letter along the same column are not significantly different ( $P \leq 0.05$ ). OH• - hydroxyl radical, DPPH - 1,1-diphenyl-2-picrylhydrazyl radical, MDA - malondialdehyde. F. harvested = Freshly Harvested mango fruit, Ref. Temp. = Mango fruit stored at refrigeration temperature, Room Temp. = Mango fruit stored at room temperature

## 4 Conclusions

The bioactive components (vitamin C, total phenolic and flavonoid) of the peel of mango remain relatively stable during cold storage of mango fruit and increased in the peel of mango fruit stored at room temperature. The peel of mango fruit stored at room temperature also exhibits higher antioxidant activities as shown in the increase in the scavenging abilities. From this work, it is concluded that freshly harvested, matured, edible and just ripe mango fruit (Sheri Mango) could be stored at room temperature and can be consumed with the peel; since storage at room temperatures has positively affected the bioactive components and enhanced the antioxidant capacity of the mango peel, and therefore the health functionality of the mango peel.

## References

- Ademiluyi, A. O. & Oboh, G. (2011). Antioxidant properties of condiment produced from fermented bambara groundnut (*Vigna Subterranea L. Verdc*). *Journal of Food Biochemistry*, 35(4), 1145–1160. doi:10.1111/j.1745-4514.2010.00441.x
- Ademosun, A. O. & Oboh, G. (2014). Anticholinesterase and antioxidative properties of water-extractable phytochemicals from some citrus peels. *Journal of basic and clinical physiology and pharmacology*, 25(2), 199–204. doi:10.1515/jbcpp-2013-0027
- Adetuyi, F. O., Ibrahim, T. A., Ajalia, L., & Oloye, D. A. (2010). Waxing effect on the physical attributes, antioxidant and sugar contents of orange (*Citrus sinensis L. osbeck*) stored at room temperature in nigeria. *Bangladesh Journal of Scientific and Industrial Research*, 45(2), 111–116. doi:10.3329/bjsir.v45i2.5707
- Ajila, C. M., Naidu, K. A., Bhat, S. G., & Rao, U. P. (2007). Bioactive compounds and antioxidant potential of mango peel extract. *Food Chemistry*, 105(3), 982–988. doi:10.1016/j.foodchem.2007.04.052
- Aruoma, O. I., Deiana, M., Rosa, A., Casu, V., Piga, R., Peccagnini, S., ... Higa, T. (2002). Assessment of the ability of the antioxidant cocktail-derived from fermentation of plants with effective microorganisms (em-x) to modulate oxidative damage in the kidney and liver of rats in vivo: studies upon the profile of poly- and mono-unsaturated fatty acids. *Toxicology Letters*, 135(3), 209–217. doi:10.1016/S0378-4274(02)00261-8
- Axelsen, P. H., Komatsu, H., & Murray, I. V. J. (2011). Oxidative stress and cell membranes in the pathogenesis of alzheimer's disease. *Physiology*, 26(1), 54–69. doi:10.1152/physiol.00024.2010
- Bapat, V. A., Trivedi, P. K., Ghosh, A., Sane, V. A., Ganapathi, T. R., & Nath, P. (2010). Ripening of fleshy fruit: molecular insight and the role of ethylene. *Biotechnology Advances*, 28(1), 94–107. doi:10.1016/j.biotechadv.2009.10.002
- Belle, N. A. V., Dalmolin, G. D., Fonini, G., Rubin, M. A., & Rocha, J. B. T. (2004). Polyamines reduces lipid peroxidation induced by different pro-oxidant agents. *Brain Research*, 1008(2), 245–251. doi:10.1016/j.brainres.2004.02.036

- Benderitter, M., Maupoil, V., Vergely, C., Dalloz, F., Briot, F., & Rochette, L. (1998). Studies by electron paramagnetic resonance of the importance of iron in the hydroxyl scavenging properties of ascorbic acid in plasma: effects of iron chelators. *Fundamental & Clinical Pharmacology*, *12*(5), 510–516.
- Bron, I. U. & Jacomino, A. P. (2006). Ripening and quality of golden papaya fruit harvested at different maturity stages. *Brazilian Journal of Plant Physiology*, *18*(3), 389–396. doi:10.1590/S1677-04202006000300005
- Cordenunsi, B. R., do Nascimento, J. R. O., Genovese, M. I., & Lajolo, F. M. (2002). Influence of cultivar on quality parameters and chemical composition of strawberry fruits grown in Brazil. *Journal of Agricultural and Food Chemistry*, *50*(9), 2581–2586. doi:10.1021/jf011421i
- De Almeida Monaco, K., Costa, S. M., Uliana, M. R., & Lima, G. P. P. (2014). Sanitizers effect in mango pulp and peel antioxidant compounds. *Food and Nutrition Sciences*, *5*, 929–935. doi:10.4236/fns.2014.510103
- Fernando, H. R. P., Srilaong, V., Pongprasert, N., Boonyaritthongchai, P., & Jitareerat, P. P. (2014). Changes in antioxidant properties and chemical composition during ripening in banana variety 'Hom Thong' (AAA group) and 'Khai' (AA group). *International Food Research Journal*, *21*(2), 749–754.
- Fujimoto, Y., Tanioka, H., Keshi, I., & Fujita, T. (1983). The interaction between lipid peroxidation and prostaglandin synthesis in rabbit kidney-medulla slices. *Biochemical Journal*, *212*(1), 167–171.
- Garcia, M., Cabral, F. A., & Martinez-Correa, H. A. (2013). Supercritical CO<sub>2</sub> and pressurized solvents extraction processes for bioactive compounds from mango peel waste (*Mangifera indica* L.). *III Iberoamerican Conference on Supercritical Fluids Cartagena de Indias (Colombia)*, 1–8. Retrieved from <http://www.nupeg.ufrn.br/prosciba/prosciba2013/Papers/T1-10.pdf>
- Gomez, M. L. P. A. & Lajolo, F. M. (2008). 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*, *88*(5), 756–762. doi:10.1002/jsfa.3042
- Gull, J., Sultana, B., Anwar, F., Naseer, R., Ashraf, M., & Ashrafuzzaman, M. (2012). Variation in antioxidant attributes at three ripening stages of guava (*Psidium guajava* L.) fruit from different geographical regions of Pakistan. *Molecules*, *17*(3), 3165–3180. doi:10.3390/molecules17033165
- Halliwell, B. & Gutteridge, J. M. C. (1981). Formation of a thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts - the role of superoxide and hydroxyl radicals. *Febs Letters*, *128*(2), 347–352. doi:10.1016/0014-5793(81)80114-7
- Imran, M., Butt, M. S., Anjum, F. M., & Sultan, J. I. (2013). Chemical profiling of different mango peel varieties. *Pakistan Journal of Nutrition*, *12*(10), 934–942. doi:10.3923/pjn.2013.934.942
- Jimenez, A., Creissen, G., Kular, B., Firmin, J., Robinson, S., Verhoeyen, M., & Mullineaux, P. (2002). Changes in oxidative processes and components of the antioxidant system during tomato fruit ripening. *Planta*, *214*(5), 751–758. doi:10.1007/s004250100667
- Kevers, C., Falkowski, M., Tabart, J., Defraigne, J.-O., Dommès, J., & Pincemail, J. (2007). Evolution of antioxidant capacity during storage of selected fruits and vegetables. *Journal of Agricultural and Food Chemistry*, *55*(21), 8596–8603. doi:10.1021/jf071736j
- Kim, Y., Brecht, J. K., & Talcott, S. T. (2007). Antioxidant phytochemical and fruit quality changes in mango (*Mangifera indica* L.) following hot water immersion and controlled atmosphere storage. *Food Chemistry*, *105*(4), 1327–1334. doi:10.1016/j.foodchem.2007.03.050
- Leong, L. P. & Shui, G. (2002). An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chemistry*, *76*(1), 69–75. doi:10.1016/S0308-8146(01)00251-5
- Lim, Y. Y., Lim, T. T., & Tee, J. J. (2006). Antioxidant properties of guava fruit: compar-

- ison with some local fruits. *Sunway Academic Journal*, 3, 9–20.
- Masibo, M. & He, Q. (2008). Major mango polyphenols and their potential significance to human health. *Comprehensive Reviews in Food Science and Food Safety*, 7(4), 309–319. doi:[10.1111/j.1541-4337.2008.00047.x](https://doi.org/10.1111/j.1541-4337.2008.00047.x)
- Meda, A., Lamien, C. E., Romito, M., Millogo, J., & Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and proline contents in burkina fasan honey, as well as their radical scavenging activity. *Food Chemistry*, 91(3), 571–577. doi:[10.1016/j.fochem.2004.10.006](https://doi.org/10.1016/j.fochem.2004.10.006)
- Moktan, B., Saha, J., & Sarkar, P. K. (2008). Antioxidant activities of soybean as affected by bacillus-fermentation to kinema. *Food Research International*, 41(6), 586–593. doi:[10.1016/j.foodres.2008.04.003](https://doi.org/10.1016/j.foodres.2008.04.003)
- Nilsson, J., Pillai, D., Onning, G., Persson, C., Nilsson, A., & Akesson, B. (2005). Comparison of the 2,2'-azinobis-3-ethylbenzotiazoline-6-sulfonic acid (abts) and ferric reducing antioxidant power (frap) methods to assess the total antioxidant capacity in extracts of fruit and vegetables. *Molecular Nutrition & Food Research*, 49(3), 239–246. doi:[10.1002/mnfr.200400083](https://doi.org/10.1002/mnfr.200400083)
- Nisha, N. & Bhatnagar, V. (2014). Utilization of mango peels as a source of phytochemicals in biscuits. *Indian Journal of Extension Education and Rural Development*, 22, 51–55.
- Nixwell, M. F., Johanna, M., & Ngezimana, W. (2013). Effects of sulphur preservative on phytochemical and antioxidant capacity of peels of mango cultivars (*Mangifera indica L.*) produced in South Africa. *African Journal of Biotechnology*, 12(41), 6007.
- Oboh, G., Ademiluyi, A. O., & Akindahunsi, A. A. (2010). The effect of roasting on the nutritional and antioxidant properties of yellow and white maize varieties. *International journal of food science & technology*, 45(6), 1236–1242.
- Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal-tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95(2), 351–358. doi:[10.1016/0003-2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3)
- Ortega, V. G., Ramírez, J. A., Velázquez, G., Tovar, B., Mata, M., & Montalvo, E. (2013). Effect of high hydrostatic pressure on antioxidant content of 'ataulfo' mango during postharvest maturation. *Food Science and Technology (Campinas)*, 33(3), 561–568.
- Oyaizu, M. (1986). Studies on products of browning reaction-antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition and Dietetics*, 44(6), 307–315. doi:[10.5264/eiyogakuzashi.44.307](https://doi.org/10.5264/eiyogakuzashi.44.307)
- Oyedoyin, B. O., Akinola, S. O., Ajuebor, F. N., Omotade, S. A., & Kupoluyi, C. F. (2008). An economic evaluation of small-scale production of clarified fruit juice from local fruits. *Journal of Industrial Research and Technology*, 2(1), 5–11.
- Palafox-Carlos, H., Yahia, E., Islas-Osuna, M. A., Gutierrez-Martinez, P., Robles-Sanchez, M., & Gonzalez-Aguilar, G. A. (2012). Effect of ripeness stage of mango fruit (*Mangifera indica L.*, cv. ataulfo) on physiological parameters and antioxidant activity. *Scientia Horticulturae*, 135, 7–13. doi:[10.1016/j.scienta.2011.11.027](https://doi.org/10.1016/j.scienta.2011.11.027)
- Perez-Jimenez, J., Arranz, S., Taberner, M., Diaz-Rubio, M. E., Serrano, J., Goni, I., & Saura-Calixto, F. (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: extraction, measurement and expression of. *Food Research International*, 41(3), 274–285. doi:[10.1016/j.foodres.2007.12.004](https://doi.org/10.1016/j.foodres.2007.12.004)
- Pinelo, M., Manzocco, L., Nunez, M. J., & Nicoli, M. C. (2004). Interaction among phenols in food fortification: negative synergism on antioxidant capacity. *Journal of Agricultural and Food Chemistry*, 52(5), 1177–1180. doi:[10.1021/jf0350515](https://doi.org/10.1021/jf0350515)
- Ramirez, J. E., Zambrano, R., Sepulveda, B., & Simirgiotis, M. J. (2014). Antioxidant properties and hyphenated hplc-pda-ms profiling of chilean pica mango fruits (*Mangifera indica L.* cv. piqueno). *Molecules*, 19(1), 438–458. doi:[10.3390/molecules19010438](https://doi.org/10.3390/molecules19010438)

- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved abts radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9-10), 1231–1237. doi:[10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3)
- Rhodes, M. J. C. (1980). The physiological-basis for the conservation of food crops. *Progress in Food and Nutrition Science*, *4*(3-4), 11–20.
- Robles-Sanchez, R. M., Islas-Osuna, M. A., Astiazaran-Garcia, H., Vazquez-Ortiz, F. A., Martin-Belloso, O., Gorinstein, S., & Gonzalez-Aguilar, A. (2009). Quality index, consumer acceptability, bioactive compounds, and antioxidant activity of fresh-cut “ataulfo” mangoes (*Mangifera Indica L.*) as affected by low-temperature storage. *Journal of Food Science*, *74*(3), S126–S134. doi:[10.1111/j.1750-3841.2009.01104.x](https://doi.org/10.1111/j.1750-3841.2009.01104.x)
- Sarkiyayi, S., Mohammed, M., & Yakubu, A. (2013). Comparative analysis of nutritional and anti nutritional contents of some varieties of mango (*mangifera indica*) in kaduna metropolis-nigeria. *Research Journal of Applied Sciences, Engineering and Technology*, *5*(4), 387–391.
- SAS. (2002). Statistical Analysis System Proprietary software. Release 8.3. SAS Institute Inc., Carry, NC.
- Shah, S. V. & Fonseca, V. A. (2011). Iron and diabetes revisited. *Diabetes Care*, *34*(7), 1676–1677. doi:[10.2337/dc11-0700](https://doi.org/10.2337/dc11-0700)
- Singh, R. P., Murthy, K. N. C., & Jayaprakasha, G. K. (2002). Studies on the antioxidant activity of pomegranate (*punica granatum*) peel and seed extracts using in vitro models. *Journal of Agricultural and Food Chemistry*, *50*(1), 81–86. doi:[10.1021/jf010865b](https://doi.org/10.1021/jf010865b)
- Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In L. Packer (Ed.), *Oxidants and antioxidants, pt a* (Vol. 299, pp. 152–178). Methods in Enzymology.
- Tachakittirungrod, S., Okonogi, S., & Chowwanapoonpohn, S. (2007). Study on antioxidant activity of certain plants in thailand: mechanism of antioxidant action of guava leaf extract. *Food Chemistry*, *103*(2), 381–388. doi:[10.1016/j.foodchem.2006.07.034](https://doi.org/10.1016/j.foodchem.2006.07.034)
- Talcott, S. T., Moore, J. P., Lounds-Singleton, A. J., & Percival, S. S. (2005). Ripening associated phytochemical changes in mangos (*Mangifera indica*) following thermal quarantine and low-temperature storage. *Journal of Food Science*, *70*(5), C337–C341. doi:[10.1111/j.1365-2621.2005.tb09963.x](https://doi.org/10.1111/j.1365-2621.2005.tb09963.x)
- Toledo-Guillen, A. R., Higuera-Ciapara, I., Garcia-Navarrete, G., & de la Fuente, J. C. (2010). Extraction of bioactive flavonoid compounds from orange (*citrus sinensis*) peel using supercritical co2. *Journal of Biotechnology*, *150*(1), S313–S314. 14th International Biotechnology Symposium and Exhibition (IBS), Rimini, ITALY, SEP 14–18, 2010. doi:[10.1016/j.jbiotec.2010.09.292](https://doi.org/10.1016/j.jbiotec.2010.09.292)
- Tunchaiyaphum, S., Eshtiaghi, M. N., & Yoswathana, N. (2013). Extraction of bioactive compounds from mango peels using green technology. *International Journal of Chemical Engineering and Applications*, *4*(4), 194–198. doi:[10.7763/IJCEA.2013.V4.293](https://doi.org/10.7763/IJCEA.2013.V4.293)
- Yusuf, S. A. & Salau, A. S. (2007). Forecasting mango and citrus production in nigeria: a trend analysis. *MPRA Paper*, (2691), 1–10. Retrieved from <https://mpra.ub.uni-muenchen.de/2691/>
- Zarena, A. S. & Sankar, K. U. (2009). A study of antioxidant properties from *Garcinia mangostana* l. pericarp extract. *Acta Scientiarum Polonorum, Technologia Alimentaria*, *8*(1), 23–34.