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# Comparison of Antioxidant Capacity of Mango (Mangifera indica), Pawpaw (Asimina triloba) and Guava (Psidium guajava) Pulp Extracts at Different Maturation Stages

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**Abstract**
The total polyphenols, flavonoids, lipid-soluble antioxidants (CALT) and radical scavenging ability of the pulp extracts of mango (*Mangifera indica*), pawpaw (*Asimina triloba*) and guava (*Psidium guajava*) were investigated at different maturation stages for the purpose of determining the antioxidant capacity and the possibility of using these fruits at every maturation stage for the prevention of lipid oxidation. The pulps of these fruits were extracted at different maturation stages; unripe (UR), about to ripe (AR) and ripe (RP). The extracted pulps were freeze-dried and used for the analyses. The total phenolic content was determined by spectrophotometry (Folic Ciocalteu's method) while 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was used for the radical scavenging ability. The various antioxidant activities were compared with standard antioxidants such as gallic acid, rutin, α-tocophenol and ascorbic acid. The results showed that all the fruits investigated at different maturation stages possessed high quality antioxidants (those that can scavenge free radicals, function as metal chelators or donate hydrogen atoms). Radical scavenging ability of the fruit pulps was significantly affected ($P < 0.05$) by the different maturation stages. The unripe fruits (mango, pawpaw and guava) have the highest antioxidant properties suggesting that the antioxidant capacity of the fruits decreased as the fruits ripened.
**Keywords:** Fruits, antioxidant capacity, maturation stage.

## 1.0 Introduction

An antioxidant is a molecule which when in small quantity are able to inhibit or prevent the oxidation of other molecules. Oxidation reactions can produce free radicals and these free radicals can initiate a chain reaction in cells and destroying the cells. Antioxidants terminate free radical chain reactions by removing free radicals, thereby controlling oxidation reactions (Sun *et al.,* 2007). A free radicals chain reaction is the major cause of lipid oxidation which is associated with cardiovascular disease and cancer. It is also the cause of rancidity of food (Grant, 2008; Fortunato *et al.,* 2007).

Antioxidants can come from natural products or be made commercially. In the past, lipid oxidation has been controlled with the use of synthetic antioxidants such as butylated hydroxy touluene, propylgallate and tert-butyl-hydroquinone (Kim *et al*., 2003). These synthetic antioxidants are suspected to be responsible for liver damage and carcinogenesis in laboratory animals (Moulisha *et al.,* 2010). It is therefore necessary to shift interest from the use of synthetic antioxidants which are harmful to the use of natural antioxidants from fruits and vegetables for the prevention of lipid oxidation.

Nigeria is blessed with fruits which are grown all over the 36 states of the country including the Federal Capital Territory (FCT). These fruits are under-utilized for antioxidant properties and should therefore be studied thoroughly to determine their antioxidant capacity.

Mango (*Mangifera indica*) belongs to the Anacardiacea family. It is considered the king of fruits (Wanthoz *et al*., 2007). The mango fruits are very popular world-wide with India having the highest production followed by China, Thailand, Mexico, Pakistan, Indonesia, the Philippines, Nigeria and Brazil (Dube *et al*., 2004). Mango fruit is a climacteric fruit which means that it may be picked when mature before ripening has commenced and subsequently ripened after post-harvest. Mango skin colour is important and can be used to determine the appropriate maturity for harvesting, processing and consumption (Cocazza *et al*., 2004). The loss of green colour is an obvious sign of ripening in many mango cultivars.

Pawpaw (*Asmina triloba*) is a member of the Annonacea family. There are more than forty named varieties of pawpaw fruit, consisting of different flavor, texture and appearance (Jones *et al.,* 1999). Pawpaw fruit contains two rows of almond- size seeds surrounded by yellow to orange- coloured flesh, with the skin ranging from green to yellow when ripe (Wiese & Duffon, 2003; Aremu & Olaofe, 2008). The fruit has high protein, carbohydrates, vitamins, minerals and essential amino acids (Peterson *et al*., 1982).

Guava (*Psidium guajava*) belongs to the family of Mystraecea. It is widely cultivated in the tropics. The genus psidium comprises about 150 species out of which the common guava (*Psidium guajava*) and pear guava (*Psidium pomeferum L*.) are some of the important species (Guangzhon, 2010). Mature guava fruit is green in colour while the ripe fruit is either white or yellow. An essential oil isolated from the leaves has shown

 

anti-cancer properties (Michelis *et al.,* 2006) while the bark extract is used for the treatment of diabetes (Bartosz, 1997). It is generally considered that different parameters such as season, variety, stages of maturity and climatic conditions are antioxidant content dependent (Hart and Scoth, 1995; Nour *et al.,* 2013).

To our knowledge, no information is available on the antioxidant capacity of pulp extracts of mango, pawpaw and guava at different maturation stages (unripe, about to ripe and ripe) grown in the southeast and Federal Capital Territory (FCT), Nigeria. Therefore, the aim of the investigation was to determine polyphenols, flavonoids, lipid–soluble antioxidants (CALT) and radical scavenging ability of the pulp extracts of mango (*Mangifera indica),* pawpaw *(Asimina triloba)* and guava *(Psidium guajava)* grown in Nigeria.

## 2.0 Materials and Methods

### 2.1 Collection of samples

Pawpaw (*Asimina triloba*) and guava *(Psidium guajava*) fruits were harvested from their trees in August, 2014 from Udi in Enugu state of Nigeria while mango (*Mangifera indica)* was harvested from Kubwa town in the FCT, Abuja, Nigeria also in August, 2014. Based on visual observations of colour, texture and flavor, the fruits were categorized into unripe (UR), about to ripe (AR) and ripe (RP). The fruits were sliced horizontally into halves with a sharp knife, seed nuts were removed for mango and guava while seed nuts, the skin and the fruit cavities cleaned for pawpaw. The categorized and sliced fruit pulps were taken to the medicinal Department of National Institute of Pharmaceutical Research and Development (NIPRD) Idu, FCT- Abuja for further treatments and analyses.

### 2.2 Preparation of samples

The categorized and sliced fruits were weighed, homogenized using a domestic blender, filtered using a cheese cloth and freeze-dried. The freeze-dried samples were stored in a sample bottle and used for analysis.

### 2.3 Chemicals and reagents

Chemicals of analytical grade (BDH) were used for the analysis. These include: Folin-Ciocalteu's phenol reagent, gallic acid, aluminium chloride, potassium acetate, 70% aqueous methanol, 1, 1-diphenyl-2-picryhydrazyl (DPPH), ascorbic acid, rutin, absolute ethanol, sulphuric acid, sodium phosphate and ammonium molybdate.

### 2.4 Total phenolic content determination

The total phenolic content was measured using Folin-Ciocalteu reagent as described by Jagadish *et al.* (2009) with some modifications. 4.0 mg of each freeze-dried categorized sample was dissolved in 4.0 ml of 70 % aqueous methanol. 0.6 ml of the methanoic extracts was mixed with 0.5 ml of 1:10 dilution of Folin-Ciocalteu phenol reagents and 0.4 ml of 0.7 M $Na_2CO_3$ solution. The solution was incubated for 10 min at a constant temperature of 100 $^oC$ in a water bath. After incubation, the solution was ice cooled and diluted with 1.5 ml distilled water and read at an absorbance of 760 nm. The total polyphenol in the fruits were obtained from a standard curve of gallic acid ranging from 0.001 to 0.5 mg/ml (Pearson's correlation coefficient: $r^2$=0.9250). Total phenol content was expressed as milligram of gallic acid equivalent (GAE) / g weight of each categorized fruit pulp tissue.

### 2.5 Measurement of total flavonoids

The total flavonoid was spectro-photometically determined as described by Grant, (2008). 1.0 mg/ml methanoic extract of each categorized sample was mixed with 10 % 0.1 ml aluminium chloride; 0.1 ml of 1.0 M potassium acetate and 2.8 ml distilled water was added. The solution was vortexed and allowed to sit for 40 min at 25$^oC$ and the absorbance read at 415 nm. The total flavonoid content of the fruits was quantified based on the standard curve prepared for rutin at concentrations of 0.004 to 1.0 mg/ml (Pearson's correlation coefficient: $r^2$=0.982). The total flavonoid was expressed as milligram of rutin equivalent/g weight of each categorized fruit pulp tissue.

### 2.6 Determination of total lipid-soluble antioxidant capacity (CALT)

A spectro-photometric method developed by Prior *et al*. (2005) was used to determine the total lipid antioxidant capacity of the fruits. 0.2 ml of 1.0 mg/ml of each freeze-dried categorized sample was dissolved in 0.2 ml absolute ethanol. The ethanoic extract was mixed with 1.0 ml of phosphomolybdenium reagent (32 mM sodium phosphate + 4 mM ammonium molybdate and 0.6 M $H_2SO_4$). The mixture was homogenized and incubated at 95$^oC$ for 90 min and the absorbance measured at 695 nm. CALT is expressed as equivalents of α-tocophenol. A Standard curve was constructed with different amounts of α-tocophenol dissolved in ethanol. An extinction coefficient E of 137 $Nm^{-1}$ ($r^2$=0.9998) was used for quantification.

CALT (mmol α-tocophenol/g fruit pulp tissue) = $A_{695}\, E^{-1}$ ERV x $SV^{-1}$ x EV x $M^{-1}$

**Where:** $A_{695}$ is the absorbance at 695 nm.

$E^{-1}$ is the inverse of the extinction coefficient.

 

RV is the overall reaction volume.
SV is the sample volume used in the reaction.
EV is the volume of solvent used in the extraction of the plant material analyzed.
M is the amount (grams) of fresh plant material.

### 2.7 Determination of radical scavenging ability

The scavenging ability of the samples on the stable free radical DPPH was evaluated using the method described by Grant (2008) with some modifications. 20.0 mg of each freeze-dried categorized samples was dissolved in 5.0 ml of 70% Methanol. The mixture was vortexed for 10 min at room temperature and filtered. Serial dilutions of the extracts were prepared at concentrations of 0.0625 to 4.0 mg/ml and 1.0 ml of 0.078 mg/ml methanol solution of DPPH was added to serial dilutions of all the extract. The mixture was allowed to stand without light for 30 min and the absorbance was measured at 517 nm. The DPPH radical scavenging effect was expressed as a percent inhibition of the DPPH radical and determined according to the equation bellow:

$$\text{Radical scavenging} = \frac{ABS\ control - ABS\ sample}{ABS\ control}\ x\,100$$

Ascorbic acid solutions of different concentrations (0.0625 to 4.0 mg/ml) were used as positive controls for antioxidant activity.

### 2.8 Statistical analysis

Data were expressed as means ± SD of three independent experiments. A one - way analysis of variance (ANOVA) was used to evaluate the significance of results. A probability $P < 0.05$ was considered significant.

## 3.0 Results and Discussion

### 3.1 Effects of total phenolic content

The total phenolic contents are essential in the assay of antioxidant capacity of fruits and vegetables (Moulisha *et al*., 2010). This is because phenolic compounds are characterized by hydroxyl groups which possess scavenging activities. It has been reported that phenolic compounds are associated with antioxidant activity and play an important role in stabilizing lipid peroxidation (Fang, 2007). Figs. 1a-c shows the mean total phenolic concentrations of unripe (UR), about to ripe (AR) and ripe (RP) mango, pawpaw and guava fruit pulps. One milligram of the unripe (UR) fruit pulps contain concentrations ranging from 0.70 to 0.86 mg of gallic acid equivalents of phenolic compounds, about to ripe (AR) ranged from 0.69 to 0.80 mg of gallic acid equivalents of phenolic compounds and ripe (RP) ranged from 0.50 to 0.84 mg of gallic acid equivalents of phenolic compounds. These concentrations of polyphenols can play an important role in antioxidant ability of these fruits. This is because it has been suggested that up to 1 g of polyphenolic compounds (from a diet rich in fruits and vegetables ingested daily has inhibitory effects on mutagenesis and carcinogenesis in humans (Moulisha *et al*., 2010). There is no significant difference in the total phenolic content of the fruits at different maturation stages, though for;

Mango fruit pulp: UR ≥ RP > AR
Guava fruit pulp: UR > AR > RP

This suggests that as the fruits ripen, the total phenolic content decreases.

 

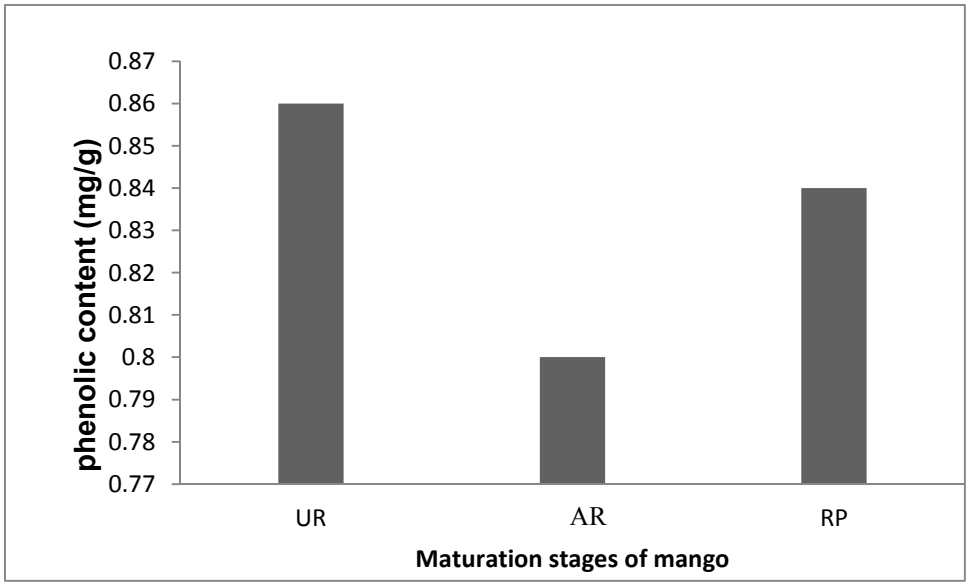


Fig. 1a: The total phenolic content of mango at different maturation stages in milligram equivalent of gallic acid/g mango pulp tissue.

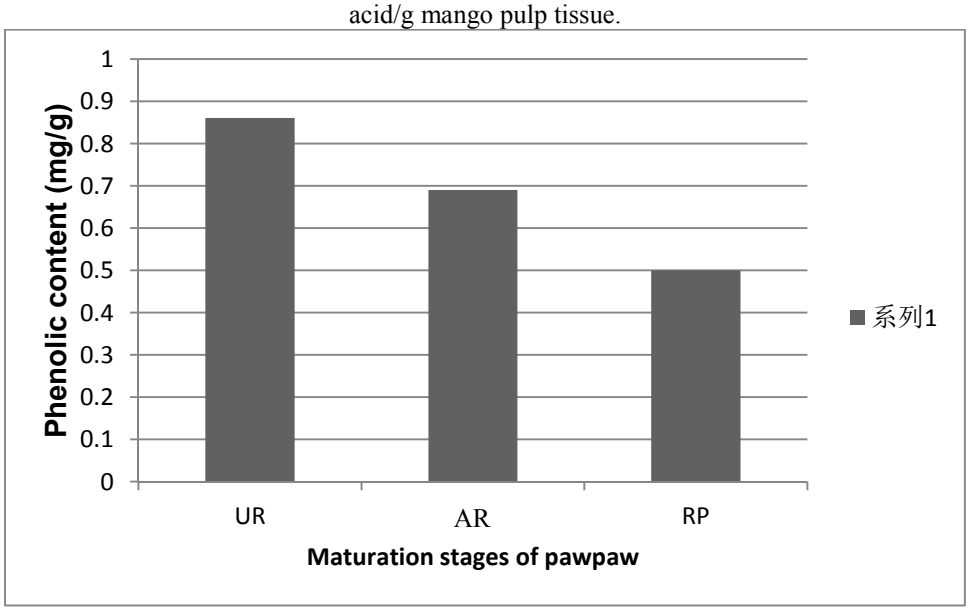


Fig. 1b: The total phenolic content of pawpaw at different maturation stages in milligram equivalent of gallic acid/g pawpaw pulp tissue.

 

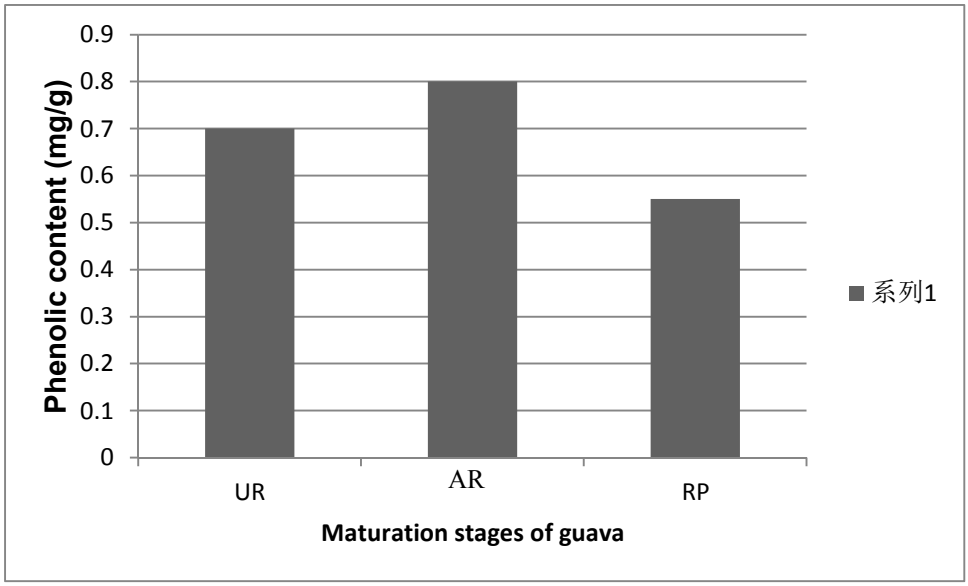


Fig. 1c: The total phenolic content of guava at different maturation stages in milligram Equivalent of gallic acid/g guava pulp tissue.

### 3.2 Effect of total flavonoids

Flavonoids are secondary metabolites which have been found to have antioxidant properties, antibacterial and antimicrobial properties (Osarumwense, 2006).

Figs. 2a-c show the mean total flavonoid concentration values for unripe (UR), about to ripe (AR) and ripe (RP) mango, pawpaw and guava respectively. The amount of total flavonoids extracted from the fruit pulps of these fruits was significantly affected by the maturation stages in the order of unripe (range 0.0463 to 0.0650 mg rutin/g tissue) > ripe (RP) (range 0.0403 to 0.0494 mg rutin/g tissue) > About to ripe (AR) (0.0153 to 0.0401 mg rutin/g tissue). The result showed that the fruits at different maturation stages were good sources of flavonoids and that the flavonoid content of the unripe fruit was highest, decreased when the fruit was about to ripe and increased again when the fruit was ripe.

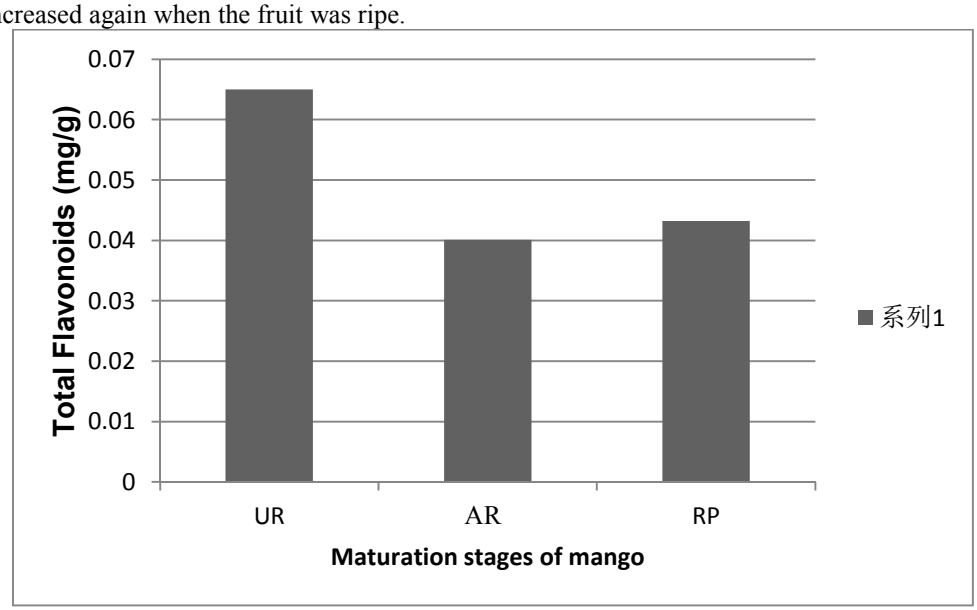


Fig. 2a: The total flavonoid content of mango at different maturation stages in milligram Equivalent of Rutin/g mango pulp tissue.

 

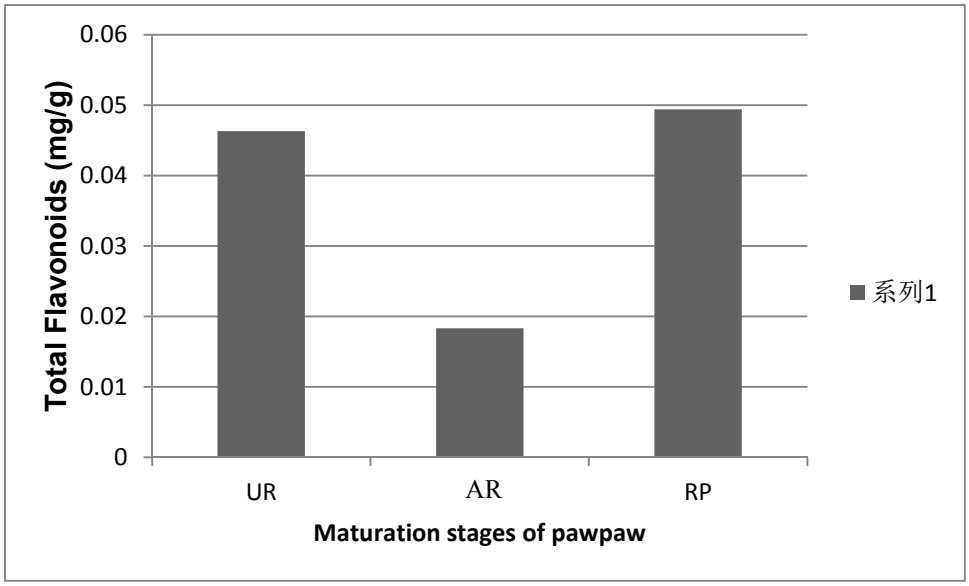


Fig. 2b: The total flavonoid content of pawpaw at different maturation stages in milligram Equivalent of Rutin/g pawpaw pulp tissue.

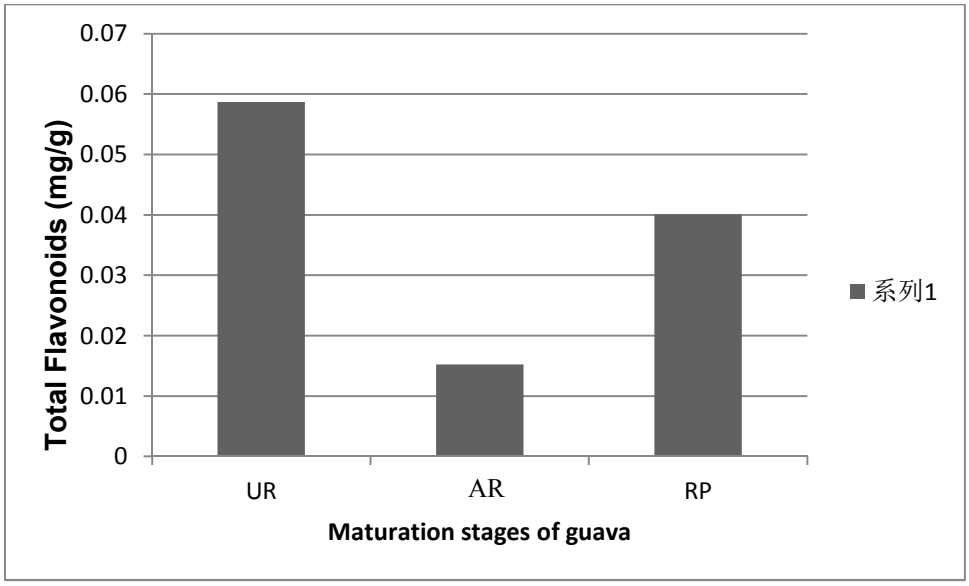


Fig. 2c: The total flavonoid content of guava at different maturation stages in milligram Equivalent of Rutin/g guava pulp tissue.

### 3.3 Effect of lipid soluble antioxidant capacity (CALT)

The lipid-soluble antioxidant capacities of the fruits were studied using α-tocophenol as a standard. There was no significant difference in the lipid soluble antioxidant capacity of the fruits at different maturation stages. Figs. 3(a-c) show the mean lipid-soluble antioxidant capacity of the fruits. The result showed that the lipid soluble antioxidant capacity of the fruits decreased as the fruit ripened. This suggests that the fruits are likely to contain more water-soluble antioxidants as they ripen.

 

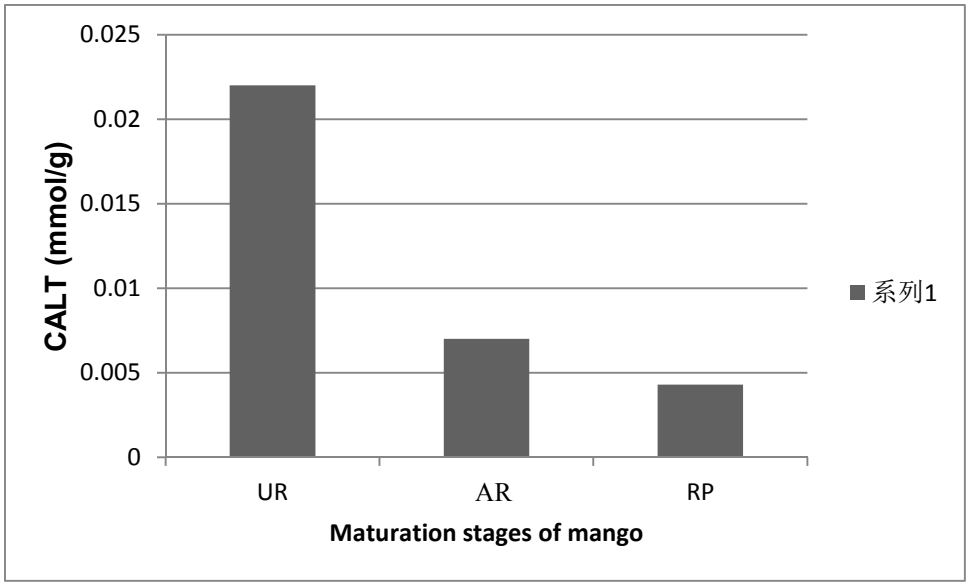


Fig. 3a: The lipid-soluble antioxidant capacity (CALT) of mango at different maturation stages in milligram Equivalent of α-tocophenol in mmol/g mango pulp tissue.

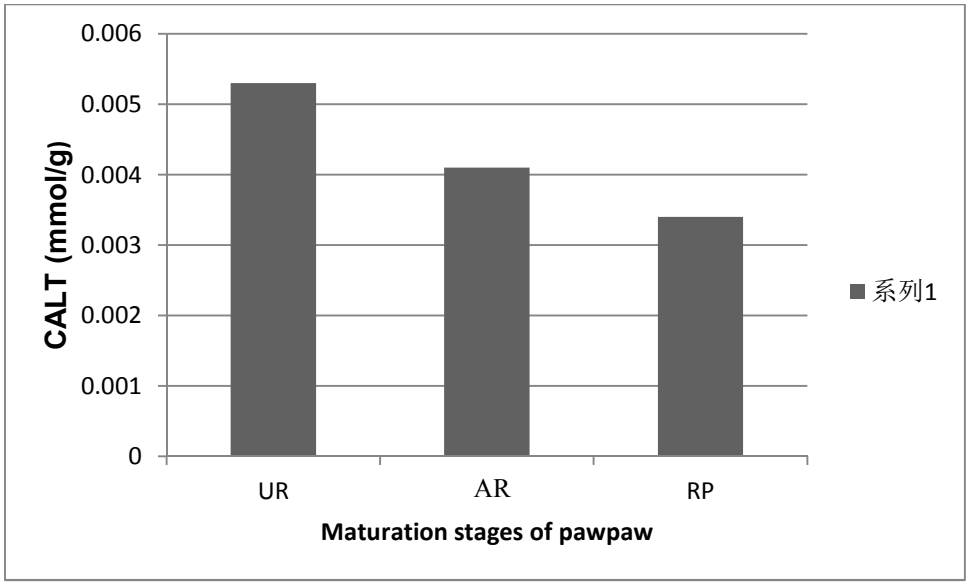


Fig. 3b: The lipid-soluble antioxidant capacity (CALT) of pawpaw at different maturation stages in milligram Equivalent of α-tocophenol in mmol/g pawpaw pulp tissue.

 

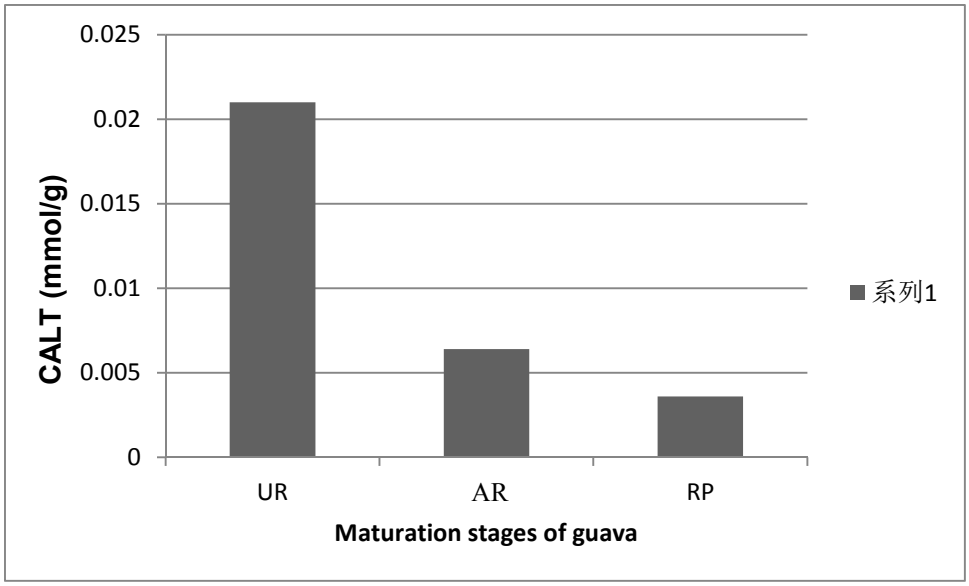


Fig.3c: The lipid-soluble antioxidant capacity (CALT) of guava at different maturation stages in milligram Equivalent of α-tocophenol in mmol / g guava pulp tissue.

### 3.4 Radical scavenging effect

The scavenging ability of an antioxidant is measured by its ability to quench the stable DPPH radical with maximum absorption at 517 nm (Grant, 2008; Lopes et al., 2012). Figs. 4 (a-c) show the DPPH free radical scavenging ability on mango, pawpaw and guava fruit pulps at different maturation stages using standard ascorbic acid for comparison. The results showed that mango, pawpaw and guava fruit pulps were good radical scavengers at different maturation stages and the scavenging abilities of these fruit pulps and ascorbic acid correlated well with increasing concentrations. The scavenging ability of the fruits increased with increasing concentration. The unripe (UR) fruit pulp has the highest scavenging activities at 4.0 mg/ml (79.5%, 71.0% and 76.6%) respectively for mango, pawpaw and guava fruit pulps. At low concentration of 0.0625 mg/ml the scavenging abilities of pawpaw (UR, AR, and RP) and about to ripe (AR) for guava were low, < 50%. The radical scavenging ability of the fruit pulps was significantly affected by the maturation stages, suggesting that the radical scavenging capacity of mango, pawpaw and guava fruit pulps decreased with ripening.

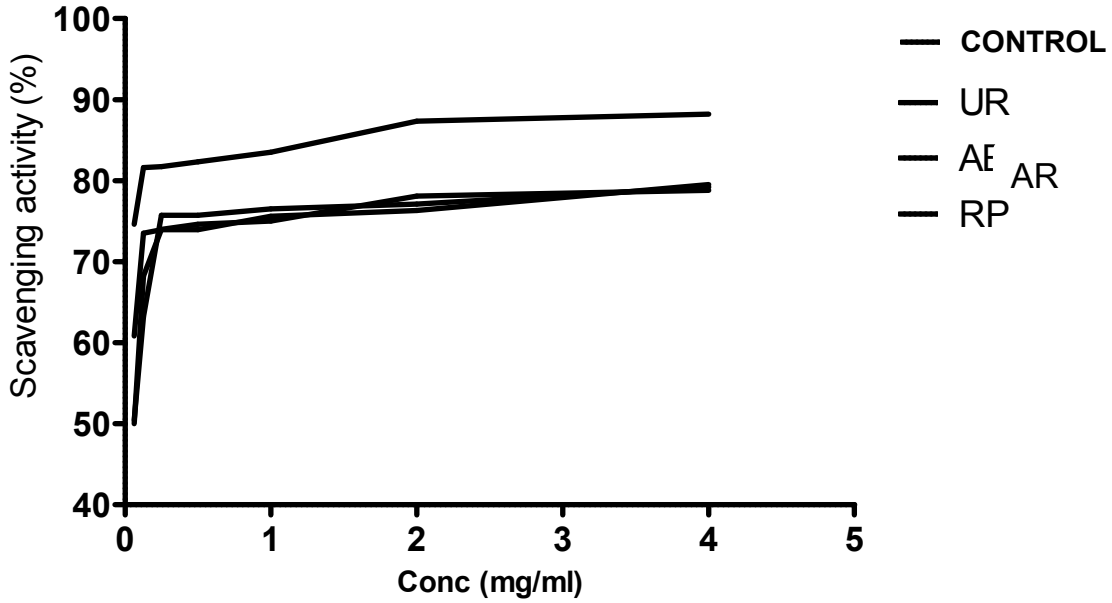


Fig 4a: The scavenging ability of mango at different maturation stages on DPPH radicals. Results are presented as means ± standard deviation (n=3). Differences are statistically significant if P<0.05 when compared to control.

 

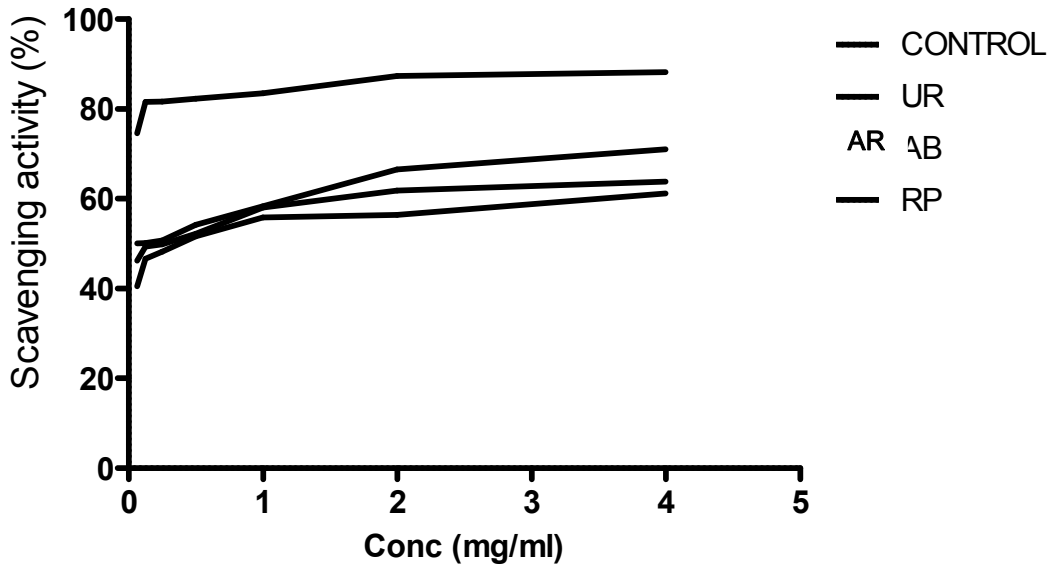


Fig 4b: The scavenging ability of pawpaw at different maturation stages on DPPH radicals. Results are presented as means ± standard deviation (n=3). Differences are statistically significant if P<0.05 when compared to control.

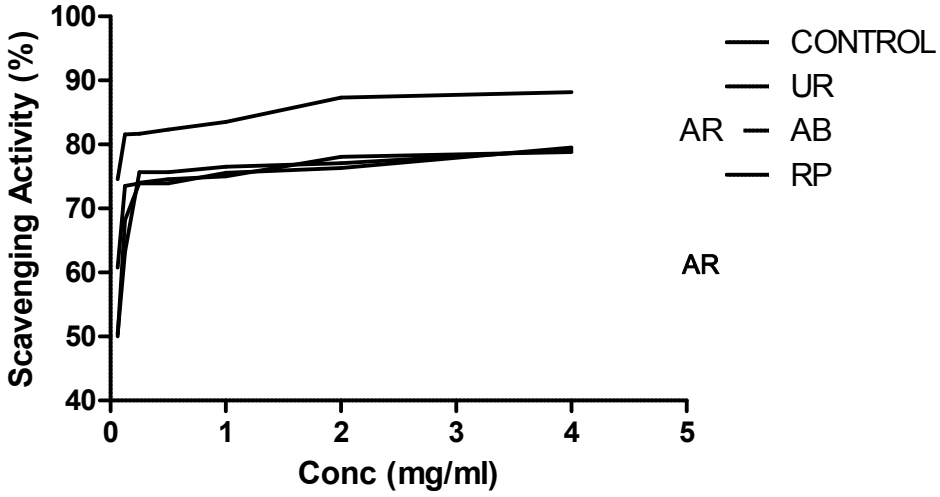


Fig 4c: The scavenging ability of guava at different maturation stages on DPPH radicals. Results are presented as means ± standard deviation (n=3). Differences are statistically significant if P<0.05 when compared to control.

## 4.0 Conclusion

The results showed that mango, pawpaw and guava fruit pulps possessed high quality antioxidants (those antioxidants that can scavenge free radicals, function as metal chelators or donate hydrogen atoms) at different maturation stages. This observation may be as a result of phytochemicals such as flavonoids and polyphenols present in the fruits. The extracts of these fruits at different maturation stages could be used to prevent lipid oxidation.

## 5.0 Acknowledgements

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## 6.0 References

Angelo, A. (1996). Lipid peroxidation in food. Critical Rev*iew Food Science & Nutrition,* 36(6), 175 – 224.

Aremu, M. O. & Olaofe, O. (2008). Fat and fatty acid composition of some plant foods found in Nigeria. A review. Indian Journal of Botanical Research, 3(2), 328–348.

Bartosz,G.(1997). Oxidative stress in plants. *Acta Physiology,* 19(1), 47-64

Cocazza, F.M., Jorge, J.T., Alves, R.E., Filgueiras, H.A., Garruti, O.S. & Pereira, M. E. (2004). Sensory and physical evaluation of cold stored "Tommy Atkins" mangoes influenced by 1-MCP and modified atmosphere packaging. Acta. 64(5), 655-661.

Dube, M., Zunker, K. & Neidhart, S. (2004). Effect of technological processing on the allegernicity of mangoes (Manifera indica L.). *Journal Agriculture, & Food Chemistry,* 52(3), 938-945

Fortunato, G. & Taranto, M. (2007). Polmorphisms and the expression of genes encoding enzymes involved in cardiovascular disease. *Clinica Chemica Acta*, 38(1), 21- 25.

Grant, G. H. (2008). Antioxidant capacity of pawpaw pulp extraction from different levels of ripeness. College of Health and Human Services. *Ohio,* 32, 48- 50.

Guang zhon, R. (2010). Antioxidant capacities and total phenolic contents of 56 wild fruits from South China. *Journal Food Nutrition & Health,* 15(8), 602-617.

Hart, D. J. & Scoth, K. J. (1995). Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. Food Chemistry, 54(1), 101–111.

Jagadish, L. K., Krishnan, V. V., Shenbnagraman, R. & Kaviyarasan, V. (2009). Comparative study on the antioxidant, anticancer and antimicrobial property of *Agaricus bisporus imbach* before and after boiling. *African. Journal Biotechnology,* 12(5), 654- 661.

Jones, S. C, Peterson, N. R., Turner, T.A., Pomper, K.W. & Layne, D.R. (1999). Pawpaw Planting Guide (PIB-002). Frankfort: Kentucky State University Cooperative Extension Program.

Kim, D. O., Jeong, S. W. & Lee, C. Y. (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry,* 81(4), 321 – 326.

Leja, M., Mareezek, A. & Ben, J. (2003). Antioxidant properties of two apple cultivars during long term storage. *Food Chemistry* 80(6), 303-307.

Lopes, M., Miranda, M., Moura, C. & Filho, J. (2012). Bioactive compound and total antioxidant capacity of cashew apples during ripening of early dwarf clones. *Journal Food Science & Technology,* 36(4), 430-500

Michelis, K. B., Giovannucci, E., Chan, A. T., Singhania, R., Fucus, C. S. & Willet, W. C. (2006). Fruits and Vegetables Consumption and Corectal Adenomas in the Nurse Health Study, 66(3), 942-953.

Moulisha, B., Halder, P. K., & Ghosh, A. K. (2010). Antioxidant and free-radical-scavenging effects of fruits of *Dregea volubilis. Journal Natural Science, Biology&Medicine,* 1(1), 29- 34.

Nour, V., Trandafir, M. E. I. & Ionica, A. (2013). Antioxidant compounds, mineral content and antioxidant activity of several tomato cultivars grown in southwestern Romania. *Notulae Botanicae Horti Agrobotanici,* 41(1), 136–142.

Osaumwense, P. O. & Okunrobo, L. (2011). Phytochemical composition of *Acalypha lispida* (EUPHORBIACEAE). *International Journal Chemical Science,* 4(1), 172 – 175.

Prior, R. L., Wu, X. & Schatech, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in food and dietary supplements. *Journal Agric and Food Chemistry,* 53(4), 290-300.

Turner, S., Cogoi, L., Isolabella, S., Filip, R. & Anesini, C. (2011). Evaluation of the antioxiodant activity and polyphenols content of *Ilex paraguariensis* (Mate) during industrialization, *Journal Food Science & Technology,* 3(1), 23-30.

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