The Influence of Storage of Pawpaw *Carica papaya* Fruit on the Bioactive Components, Antioxidative Properties and Inhibition of Fe^{2+}-Induced Lipid Peroxidation of Water-Extracts of Pawpaw Seed

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Abstract. Freshly harvested, matured, newly ripe pawpaw (*Carica papaya*) fruit (Maradol variety) was stored at room temperature (27±1 °C) for 7days. The seeds of the freshly harvested and stored pawpaw fruit were taken and divided into two groups each; one was dried as dry sample, dry freshly harvested sample (DFHS) and dry stored sample (DSS) while the other was left as wet sample, wet freshly harvested sample (WFHS) and wet stored sample (WSS). The bioactive components, antioxidative properties and inhibition of Fe^{2+} induced lipid peroxidation activity of the water extract of the seed were investigated. Storage of pawpaw fruit caused a significant increase in ascorbic acid and flavonoid content of the pawpaw seed in both dry and wet samples but only in dry stored sample significant increase in the phenol content of the seed was observed. The dried pawpaw seed DFHS and DSS showed stronger inhibition of Fe^{2+} induced lipid peroxidation activities.

Keywords: pawpaw seed, storage influence, phenol, ascorbic acid, flavonoid, DPPH scavenging ability, lipid peroxidation

Introduction

Pawpaw (*Carica papaya*) is a small tropical tree native to South America. It normally grows with a single and branched trunk which may reach 10 m in height but is more commonly 4-5 m tall. The pawpaw plant is widely spread throughout tropical Africa. It belongs to the group *Caricaceae* (Adetuyi *et al*., 2008). The skin colour of pawpaw fruit is usually green when immature, changing to fully reddish-orange or yellow-orange when fully ripened. The changes in the outer colour of the fruit skin is an indication of ripeness, and this change is considered mainly due to an increase in the carotene content and a decrease in chlorophyll (Maisarah *et al*., 2014). In the early stages of fruit development, the main sugar in pawpaw is glucose. There is an increase in sucrose content of pawpaw fruit during ripening which can be up to 80% of the total sugars. The crude protein content has been reported to range from 3.74-8.26 g/ 100 g dry matter while aspartic and glutamic acid are the most abundant amino acids in ripe fruits (Saran and Choudhary, 2013).

The central cavity of pawpaw fruit contains large quantities of seeds that are small, black, round, covered

with gelatinous aril and make up about 15% of the wet weight of the fruit (Malacrida *et al*., 2011; Afolabi and Ofobrukweta, 2011). Several studies revealed that pawpaw seeds are a rich source of proteins (27.3-28.3%), lipids (28.2-30.7%), and crude fibres (19.1-22.6%). Also it contains appreciable quantities of calcium and phosphorus (Malacrida *et al*., 2011). In pawpaw seed the enzyme carpesemine is a plant growth inhibitor and oleanolic glycoside is an agent of sterility in male albino rats. Alkaloids have been reported in the endosperm of pawpaw seed. Fatty acids such as oleic, palmitic, stearic and linoleic acid have been found in pawpaw seed (Bolu *et al*., 2009). It has been discovered that glutamic acid, arginine, proline, and aspartic acid are present in the endosperm of pawpaw seed while proline, tyrosine, lysine, aspartic acid and glutamic acid are found in the sarcotesta (Saran and Choudhary, 2013).

Pawpaw latex, shoot, leaves, fruits and seeds contain a broad spectrum of phytochemicals including enzymes, carotenoids, alkaloids, phenolics, and glucosinolates. People in Lao, Cambodia, and Vietnam use the latex to treat eczema and psoriasis. The seeds have been used as vermifuge, thirst quencher, or pain alleviator (Krishna *et al*., 2008; Amenta *et al*., 2000). Phenolic compounds

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in pawpaw fruit peel and seeds have antioxidative properties (Ang *et al*., 2012). The seed of pawpaw has antimicrobial activity against *Trichomonas vaginalis* trophozoites. The seeds, irrespective of its fruit maturity stages have bacteriostatic activity on gram positive and gram negative organisms which could be useful in treating chronic skin ulcer (Afolabi and Ofobrukweta, 2011).

Pawpaw seed has shown clearly to have antihelminthic, immunomodulatory activities and having anti-amoebic properties; they are also rich source of amino acids especially in the sarcotesta. Air dried papaya seeds with honey showed significant effect on human intestinal parasites without significant side effect (Saran and Choudhary, 2013; Okeniyi *et al*., 2007). The pawpaw seed extract has shown to possess gastro-protective effect on ethanol induced gastric ulcer in rats and also used in treatment of sickle cell disease (Saran and Choudhary, 2013; Okewumi and Oyeyemi, 2012). Seeds of overripe pawpaw fruit are good source of protein, energy, nutritive minerals and have low antinutrient values (Oyeleke *et al*., 2013).

There has been no previous study to directly compare bioactive contents and antioxidant properties of pawpaw seed, with respect to storage of pawpaw fruit. Therefore, the aim of this study was to determine the effect of storage of pawpaw fruit at room temperature on the bioactive content and antioxidant properties of pawpaw seed.

Materials and Methods

Materials. The maturity stages of the pawpaw used in this study were evaluated visually based on the skin colour as defined by Hana and Angelo (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, newly ripe pawpaw fruit (Maradol variety) stage 1 with slight appearance of yellow colour obtained from Ondo State University of Science and Technology farm was used for this study.

Reagents. Ascorbic acid was from Merck (Darmstadt, Germany), gallic acid and quercetin were from Aldrich (Steinheim Germany), 1,1-diphenyl-2 picrylhydrazyl (DPPH), 1,10 orthophenanthroline, trichloroacetic acid (TCA) and potassium ferricyanide were obtained from Sigma Chemical Inc. (St Louis, MO, USA). Glass-distilled water and analytical grade chemicals and solvent were used in the present study.

Sample preparation. The freshly harvested pawpaw fruit was stored at room temperature (27±1 ºC) for 7 days. The seed of the freshly harvested and stored pawpaw fruit (200 g each) were taken and divided into two groups; one was dried in the oven at 45 °C for 5 days as dry sample, dry freshly harvested sample (DFHS) and dry stored sample (DSS) while the other was left as wet sample, wet freshly harvested sample (WFHS) and wet stored sample (WSS). The ascorbic acid, total phenol, flavonoid, ABTS scavenging ability, ferric reducing antioxidant property (FRAP), Fe^{2+} chelation assay, DPPH free radical scavenging ability and lipid peroxidation of the dry and wet samples were determined. All determinations were done in triplicates.

Aqueous extract preparation. The aqueous extracts of pawpaw seeds was prepared using a modified procedure described by Oboh *et al*. (2010). Ten grams (10 g) of the dry pawpaw seed and wet pawpaw seed were separately homogenised in 100 mL distilled water in a Warring blender. The homogenate was centrifuged at 4500 g for 15 min. The supernatant was used for the determination of the bioactive content and antioxidant properties of pawpaw seed.

Ascorbic acid content determination. The ascorbic acid 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 $CuSO_4$. $5H_2O$ in 100 mL of 5 mL H_2SO_4) was 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 h at 37 °C, then 0.5 mL of 65% H_2SO_4 (v/v) was added to the medium and the absorbance was measured at 520 nm in a spectrophotometer (JENWAY 6305). The vitamin C content of the extracts was subsequently calculated using ascorbic acid as standard.

Phenolic content determination. The total phenol content was determined according to the method of Singleton *et al*. (1999). Appropriate dilutions of the extracts were mixed with 2.5 mL of 10% Folin-Ciocalteau'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 standard.

Flavonoid content determination. The total flavonoid content of the extracts was determined using a slightly modified method reported by Meda *et al*. (2005). Briefly, 0.5 mL of appropriately diluted sample was mixed with 0.5 mL methanol, 50 mL of 10% aluminium chloride ($AlCl_3$), 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 standard.

Ferric reducing antioxidant power (FRAP). The reducing property of the extracts was determined by assessing the ability of the extract to reduce $FeCl_3$, 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 standard.

Iron chelating activity. Iron chelating activity was determined according to the method of Kuda *et al*. (2005). The pawpaw sample solution (0.1 mL) was taken into 10 mL test tube, 0.1 mL of distilled water and 0.025 mL of 2.5 mM iron chloride were added, it was thoroughly shaken and the absorbance was taken at 550 nm (Abs1). 0.025 mL of 2.5 mM ferrozine was added to the mixture, it was allowed to stay for 20 min at room temperature; the absorbance was taken at 550 nm (Abs2).

DPPH free radical scavenging ability. The free radical scavenging ability of the extract was determined using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) as described by Singh *et al*. (2002). Different concentrations of the aqueous extract were taken in different test tubes and the volume was prepared 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:

> Free radical scavenging activity (%) = (control OD – sample OD)/control OD×100

ABTS scavenging ability. The ABTS scavenging ability was determined according to the method described by Re *et al*. (1999). The ABTS was generated by reacting an ABTS aqueous solution (7 mM/L) with potassium persulphate ($K_2S_2O_8$) (2.45 mM/L, final concentration) in the dark at room temperature for 16 h and adjusting the absorbance at 734 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 min in the JENWAY uv - visible spectrophotometer. The trolox equivalent antioxidant capacity was subsequently calculated.

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 rapidly isolated and placed on ice and weighed. This tissue was 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 mins at 3000 × g to yield a pellet that was discarded, and a low – speed supernatant (SI) which was kept for lipid peroxidation assay (Belle *et al*., 2004).

Lipid peroxidation and TBA reactions. The lipid peroxidation assay was carried out using the modified method of Ohkawa *et al*. (1979). Briefly, 100 µL SI fraction was mixed with a reaction mixture containing 30 µL of 0.1M Tris-HCl buffer (pH 7.4), the extract (0 - 100 µL) and 30 µL of 250 µM freshly prepared $FeSO_4$. The volume was made up to 300 µL by water before incubation at 37 °C for 1 h. The colour reaction was developed by adding 300 µL of 8.1% sodium dodecyl sulphate (SDS) to the reaction mixture containing SI; this was subsequently followed by the addition of 600 µL acetic acid/HCl (pH 3.4) mixture and 600 µL of 0.8% thiobarbituric acid (TBA). This mixture was incubated at 100 °C for 1 h. 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 standard curve using malondialdehyde (MDA).

Statistical analysis. The results of the three replicates were pooled and expressed as mean ± standard deviation. Standard deviations were calculated using spread sheet software (Microsoft Excel©, 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 = 0.05. IC_{50} (extract concentration causing 50% inhibition of antioxidant activity) was calculated using the linear regression analysis.

Results and Discussion

Ascorbic acid. The ascorbic acid, total phenol and flavonoid contents of the extract of pawpaw seed are presented in Table 1. The ascorbic acid content of the extract ranged from 27.14 mg ascorbic acid equivalent (AAE/100 g) wet freshly harvested sample (WFHS) to 37.14 mg AAE/100g wet stored sample (WSS). The value of ascorbic acid reported in this work was higher than that reported for papaya seed but lower to the ascorbic acid of papaya pulp and peel (Maisarah *et al*., 2014; Santos *et al*., 2014; Nwofia *et al*., 2012). The result showed that the ascorbic acid content of wet sample extracts (WFHS and WSS) was significantly higher than the corresponding dry sample extracts (DFHS and DSS). This could be because ascorbic acid is easily destroyed by heat. The ascorbic acid content of the seed of stored pawpaw fruit in both wet and dry samples was significantly higher than the seed of freshly harvested fruit. The high ascorbic acid content of the seed of stored pawpaw fruit could be as a result of ripening since the pawpaw fruit stored at room temperature has ripened more than the freshly harvested pawpaw fruit. Ripening has been observed to cause increase in vitamin C content of guava and mango (Ortega *et al*., 2013; Gull *et al*., 2012). The increase in ascorbic acid contents of the seed of stored pawpaw fruit as a result of 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 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).

Phenolic content. The levels of total phenol content in the seed of the pawpaw fruit varied significantly from

Table 1. Phenol, flavonoid, nonflavonoid and ascorbic acid content of pawpaw seed

Pawpaw sample	Phenol (mg/GAE/g)	Flavonoid (mg/QE/g)	Non flavonoid (mg/QE/g)	Ascorbic acid (mg/AAE/ 100 g)
WFHS	25.71±0.00[c]	3.16±0.00[d]	22.55±2.33[c]	35.72±2.02[b]
DFHS	34.29±0.00[b]	5.79±0.00[b]	28.50±1.89[b]	27.14±6.05[d]
WSS	25.24±0.67[c]	3.95±0.36[c]	21.29±0.00[d]	37.14±0.00[a]
DSS	41.43±0.67[a]	7.63±0.37[a]	33.80±2.17[a]	34.29±0.00[c]

Values represent mean ± standard deviation of triplicate determination. Values with the same letter along the same column are not significantly different (P ≤ 0.05). GAE = gallic acid equivalent; AAE = ascorbic acid equivalent; QE = quercetin equivalent; WFHS = wet freshly harvested sample, DFHS = dry freshly harvested sample, WSS = wet stored sample, DSS = dry stored sample.

25.24 to 41.43 mg gallic acid equivalent (GAE/g). The dry seed of stored pawpaw fruit DSS contained the highest phenolic content (41.43 mg GAE/g), followed by the dry seed of freshly harvested pawpaw fruit DFHS (34.29 mg GAE/g), wet seed of freshly harvested pawpaw fruit WFHS (25.71 mg GAE/g) and wet seed of stored pawpaw fruit WSS (25.24 mg GAE/g). The phenolic content reported in this work was higher than the value reported in previous study for papaya seed on dry weight basis (Maisarah *et al*., 2014; Santos *et al*., 2014) but lower to 211.31 g GAE/g and 156.53 g GAE/g reported for water and hexane extract of papaya seed (Kothari and Seshadri, 2010). The result indicated that the dry seed of pawpaw fruit (DFHS and DSS) contain high phenolic content in both freshly harvested and stored pawpaw fruit, which may provide good sources of dietary antioxidant and gastro protective medicine. The phenolic content of the wet samples of pawpaw seed decreased in storage but not significant. The stored pawpaw fruit has ripened in storage than freshly harvested which could be a factor for the decrease in the phenolic content. The findings of Gull *et al*. (2012) in ripening stages of guava also showed that there was an apparent gradual decrease in the total phenolic contents during ripening of guava fruit, which might be associated with an amplified polyphenol oxidase activity. Ripening and other 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 subse-quent processing operations (Rhodes, 1980).

Flavonoid content. The result of Flavonoid in mg quercetin equivalent (QE/g) revealed that dry seed of pawpaw fruit at both freshly harvested and stored fruit had a significantly higher flavonoid (7.63 mg QE/g (DSS) and 5.79 mg QE/g (DFHS) content than wet seed of pawpaw fruit (3.95 mg QE/g (WSS) and 3.16 mg QE/g (WFHS)). The flavonoid in the present study was within the range of values reported by Kothari and Seshadri (2010) for papaya seed using ethanol and water extraction method 10.20-4.42 mg QE/g but higher than 59.54 mg/100 g reported by Maisarah *et al*. (2013). There was an increase in the flavonoid content of pawpaw seed due to storage. The basis of the increase could not be categorically stated; though, it may be due to ripening, a physiological activity that releases ethylene, a phytohormone that stimulates the activity of the key enzyme involved in polyphenol biosynthesis, phenylalanine ammonium lyase (PAL), which leads to formation of polyphenols. PAL activity has been shown to have a direct influence on total flavonoids (Matthes and Schmitz-Eiberger, 2009). An increase in polyphenol content during shelf life has been explained to be caused by a higher ethylene production which resulted in a stimulation of PAL (Napolitano *et al*., 2004). The non-flavonoid content of the pawpaw seed of freshly harvested pawpaw fruit and stored pawpaw fruit followed the same observation for both the total phenol and flavonoid contents where the values for the dry seeds of pawpaw fruit were significantly higher than others in the order of DSS > DFHS > WFHS > WSS (Table 1).

In complex systems, like food and food preparations, there are different mechanisms that may contribute to oxidative processes, in some mechanism, transition metal ions play a vital role, where different ROS may be generated and molecules like lipids, proteins and carbohydrates may be affected, while in another mechanism the ability of the extracts to donate electrons or the ability to protect carbohydrate structures or the ability to chelate Fe^{2+} is tested (Hinneburg *et al*., 2006; Halliwell, 1997). Several food components like carotenoids, vitamin C and E, polyphenolic compounds and the synergisms among them contribute to the overall antioxidant ability of food, it will then be difficult to assess total antioxidant ability on the basis of these individual active components alone (Pinelo *et al*., 2004). It is on this premise that ferric reducing antioxidant power (FRAP), iron chelating activity, 1,1-diphenyl-2 picrylhydrazyl (DPPH) radical scavenging abilities,

2,2-azinobis-3-ethylbenzo-thiazoline-6-sulphonate (ABTS), lipid peroxidation and thiobarbibutric acid reactions (TBARS) were evaluated for the measurement of total antioxidant activity of pawpaw seed in both freshly harvested and stored pawpaw fruit.

Ferric reducing antioxidant power (FRAP). Iron (III) ion Fe^{3+} reduction has been used many times as an indicator of electron- donating activity, which is an important mechanism of phenolic antioxidant action (Hinneburg *et al*., 2006). The Fe^{3+} to Fe^{2+} reducing activity of the pawpaw seed is expressed as mg Ascorbic Acid Equivalents AAE/g sample. Figure 1 shows that all the extracts have the ability to reduce Fe^{3+} to Fe^{2+}. The best activity was found in dry seed of stored pawpaw fruit DSS (14.28 mg AAE/g) which was significantly higher than the other extracts. There was no significant difference in the FRAP activity of the wet seed extract of freshly harvested pawpaw fruit WFHS and wet seed extract of stored pawpaw fruit WSS, the wet seed extracts performed significantly lower than the dry seed extracts in both the freshly harvested and stored pawpaw fruit. The reducing power of pawpaw seed extracts from the FRAP results demonstrated a potential antioxidant activity in consumer's body. Such a remarkable high reducing power in the dry seed of stored pawpaw fruit (DSS) was attributed to their high phenolic and flavonoid content. FRAP assay has been considered to be a good
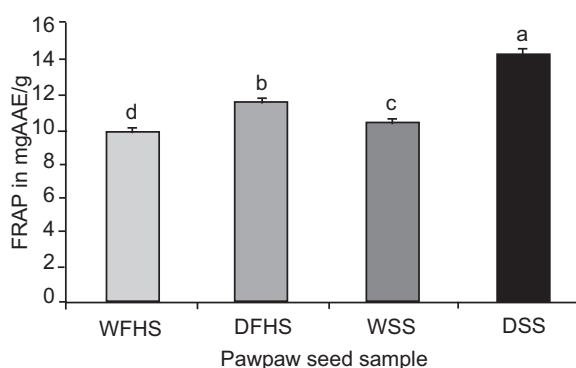
Fig. 1. Ferric reducing antioxidant property (FRAP) of pawpaw seed.
Values represent mean ± standard deviation of triplicate determination. Values with the same letter are not significantly different (P ≤ 0.05). AAE – Ascorbic Acid. WFHS = wet freshly harvested sample, DFHS = dry freshly harvested sample, WSS = wet stored sample, DSS = dry stored sample.

method in evaluating the antioxidant capacity of fruits (Palafox-Carlos *et al*., 2012). However, it was discovered that FRAP have some disadvantages in that any electron-donating substances with redox potential lower than that of the redox pair Fe^{3+}/Fe^{2+} can contribute to the FRAP value and indicate a false high values of FRAP even if they don't have antioxidant properties (Nilsson *et al*., 2005).

Iron chelation. Foods are always contaminated with transition metal ions and these may be introduced by processing methods. Bivalent transition metal ions have a crucial role to play as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions via Fenton chemistry (Halliwell, 1997). These can be prevented by iron chelation and deactivation. Therefore, the ability of the extracts of the pawpaw seed to chelate Fe^{2+} ions was evaluated and results are presented in Table 2. The seed of stored pawpaw fruit in the dry sample (DSS) and wet sample (WSS) showed the best iron chelating ability of 25.65%, respectively, which was significantly higher than others; it was closely followed by the dry seed of freshly harvested pawpaw fruit (24.36%). The basis for the high chelating ability of WSS 25.65% could not be categorically stated; however, it is expected that DSS would have a high chelation activity because of its high phenolic and flavonoid content which has been positively correlated to the iron chelation activity of food (Al-Farga *et al*., 2014; Hinneburg *et al*., 2006).

The DPPH. The DPPH results were used to confirm the results obtained in total phenolic content (Palafox-Carlosa *et al*., 2012). DPPH radical-scavenging ability

Table 2. Fe^{2+} chelation, ABTS, IC_{50} of DPPH and lipid peroxidation of pawpaw seed

Pawpaw sample	Fe^{2+} chelation (%)	ABTS (mmol.TEAC/ 100 g)	DPPH IC_{50}(mg/ mL)	Lipid peroxi-dation IC_{50}(mg/mL)
WFHS	21.80 ± 5.43^{c}	2.08 ± 0.27^{c}	33.66 ± 4.12^{b}	11.28 ± 1.00^{a}
DFHS	24.36 ± 1.81^{b}	7.17 ± 0.53^{a}	15.36 ± 1.81^{d}	4.5 ± 0.00^{d}
WSS	25.65 ± 3.62^{a}	0.94 ± 0.26^{d}	38.60 ± 3.76^{a}	6.53 ± 0.64^{b}
DSS	25.65 ± 3.63^{a}	6.04 ± 0.53^{b}	15.08 ± 1.67^{c}	5.68 ± 0.63^{c}

Values represent mean ± standard deviation of triplicate determination. Values with the same letter along the same column are not significantly different (P ≤ 0.05). TEAC = trolox equivalent antioxidant capacity; WFHS = wet freshly harvested sample; DFHS = dry freshly harvested sample; WSS = wet stored sample; DSS = dry stored sample.

of the pawpaw seed at 0-33.33 mg/mL concentrations was measured and the results are presented in Fig. 2. A dose-response relationship was found in the DPPH radical-scavenging ability of the pawpaw seed extracts; the ability increased significantly with an increase in the concentration of the pawpaw seed extracts. It showed from these results that the dry seed of pawpaw fruit in both freshly harvested and stored pawpaw fruit (DSS and DFHS) recorded the highest DPPH radical-scavenging ability which was significantly higher than the wet seed of pawpaw fruit (WFHS and WSS). It can be seen from Table 2 that DSS with the lowest IC_{50} (15.08 mg/mL) exhibited the highest DPPH radical-scavenging activity, followed by DFHS (IC_{50} 15.36 mg/mL). These result suggested that dry pawpaw seed (DSS and DFHS) are competent DPPH radical scavenger. This is an indication that it has the ability to protect consumer's health from various free-radical related diseases including ageing, cancer, atherosclerosis, Alzheimer disease, diabetes and so on.
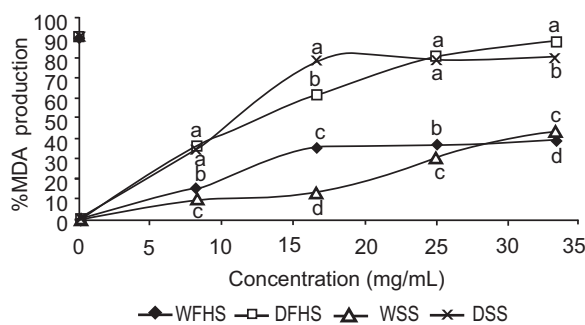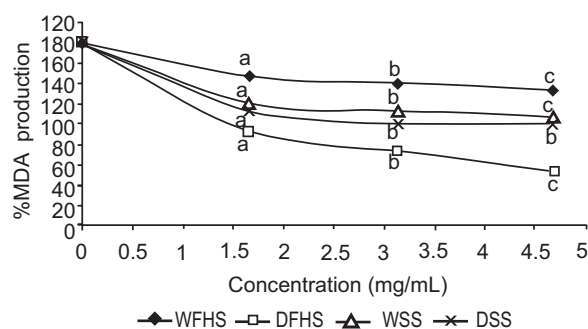

Fig. 2. DPPH scavenging abilities of pawpaw seed. Values represent mean ± standard deviation of triplicate determination. Values with the same letter along the same column are not significantly different (P ≤ 0.05). WFHS = wet freshly harvested sample, DFHS = dry freshly harvested sample, WSS = wet stored sample, DSS = dry stored sample.

The ABTS. The ABTS radical has been used to confirm results obtained with DPPH, because both possess similar antioxidant mechanisms. The versatility of ABTS radical scavenging ability is an advantage since it can measure both non-polar and polar antioxidants and spectral interference is minimized as the absorption maximum used is 760 nm, a wavelength not normally encountered with natural products (Perez-Jimenez *et al*., 2008; Re *et al*., 1999).

ABTS scavenging ability reported as trolox equivalent antioxidant capacity (TEAC) of the pawpaw seed of freshly harvested pawpaw fruit and stored pawpaw fruit is presented in Table 2. The result revealed that the ABTS scavenging ability of dry seed of freshly harvested pawpaw fruit DFHS 7.17 mmol. TEAC/100 g was significantly higher than other samples, closely followed by the dry seed of stored pawpaw fruit DSS 6.04 mmol. TEAC/100 g. The higher the TEAC value of the sample, the stronger the antioxidant activity (Zarena and Sankar, 2009). It is to be noted that high antioxidant activities in extracts may be attributed to the phenolic and flavonoid content of the extract, it was expected considering the high phenolic and flavonoid content of the extract of dry seed of stored pawpaw fruit (DSS) to have the highest ABTS scavenging ability but contrary is the case in this work where ABTS scavenging ability observed do not follow the order of phenolic and flavonoid content.

Lipid peroxidation. 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 to the development of Alzhemier's disease (Axelsen *et al*., 2011). The protective capacity of the pawpaw seed extracts against Fe^{2+} induced lipid peroxidation in cultured rat brain, is shown in Fig. 3. Incubation of rat's brain in the presence of 250 µM iron (II) caused a significant increase in the malondialdehyde (MDA) content of the brain (180%). The increase in iron in this organ resulted in the formation of reactive oxygen species ROS which facilitates lipid peroxidation through Fenton reaction. This could have accounted for the increase in MDA contents of the brain after incubation in the presence of Fe^{2+}. The result showed that the aqueous extracts of pawpaw seed significantly inhibited MDA production in the brain in a dose dependent manner. The dry seed of freshly harvested pawpaw fruit and stored pawpaw fruit had the highest inhibitory effect on the Fe^{2+} induced lipid peroxidation in rat brain since they exhibited the least IC_{50} 4.05 mg/mL DFHS and 5.68 mg/mL DSS (Table 2). The lipid peroxidation inhibition in this work followed the trend observed for ABTS scavenging ability where the scavenging ability of the dry seed of freshly harvested pawpaw fruit (DFHS) was significantly higher than other samples, closely followed by the dry seed of stored pawpaw fruit (DSS). The possible mechanism through which the extracts protect the brain could be by Fe^{2+} chelation and the scavenging of hydroxyl ion (Ademosun and

Oboh, 2014). The ability of pawpaw seed extracts to inhibit Fe^{2+} induced lipid peroxidation in rat brain homogenate *in-vitro* is a reflection that pawpaw seed extracts could prevent the elevation of brain iron levels which can cause increase in iron transport across the blood brain barrier and resulted to extracellular iron overload of the brain that can lead to neurodegenerative condition of Alzhemier's disease.

Fig. 3. Inhibition of Fe^{2+} induced MDA production in rat brain by pawpaw seed extract.

Values represent mean ± standard deviation of triplicate determination. Values with the same letter along the same column are not significantly different (P ≤ 0.05). WFHS = wet freshly harvested sample, DFHS = dry freshly harvested sample, WSS = wet stored sample, DSS = dry stored sample.

Conclusion

The total phenol and flavonoid content of pawpaw seed in dry samples increased when pawpaw fruit was stored but storage of pawpaw fruit has no significant effect on the total phenol and flavonoid content of pawpaw seed in wet samples. Storage of pawpaw fruit caused an increase in the ascorbic acid content of pawpaw seed in both wet and dry samples. Storage of pawpaw fruit also caused an increase in the scavenging abilities of dry pawpaw seed since the dry pawpaw seed of stored pawpaw fruit exhibited higher antioxidant activities. The knowledge about the antioxidant composition of the seeds of stored pawpaw fruit when dried provides important information for pharmaceutical and food industries interested in adding value to pawpaw fruit.

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