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*Full Length Research Paper*

# A comparative study on ripe and unripe eggplant (*Solanum melongena*) as dietary antioxidant sources

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**Varieties of eggplants are consumed in the diets around the world and have been reported to possess antioxidant potentials. However, the present study was designed to evaluate the effect of ripening on the antioxidant properties of eggplant. *In vitro* chemical measures of antioxidant activity employed are DPPH radical scavenging, reducing properties, iron chelation, prevention of both deoxyribose degradation and lipid peroxidation by the seed and flesh of both ripe and unripe eggplant. In addition, the phenol and flavonoid contents of both the ripe and unripe eggplant were also evaluated. The results indicate that ripening increases the radical scavenging ability and reducing properties of the flesh, whereas the iron chelating ability of the flesh was unaltered. However, both flesh and seed of ripe and unripe eggplant exerted similar inhibitory effect on the $Fe^{2+}$ and $H_2O_2$-induced deoxyribose degradation and hepatic lipid peroxidation induced by both $Fe^{2+}$ and sodium nitroprusside. Hence, we concluded that when consumed raw, both ripe and unripe eggplant can be considered as potential antioxidant sources.**

**Key words:** Eggplant (*Solanum melongena*), antioxidant property, oxidative stress.

## INTRODUCTION

A number of studies have reported that diet is a highly important factor in terms of management of degenerative diseases in which free radical-induced oxidative stress have been implicated in their etiology. It is already known that some dietary micronutrients, like flavonoids, carotenoids, and others, can play an important role in the modulation or prevention of free radical induced degenerative diseases such as cancer and diabetes. In this regard, eggplant (*Solanum melongena*) fruits, which has been reported to contain ascorbic acid and phenol-lics, both of which are powerful antioxidants (Vinson et al., 1998; Kwon et al., 2008), has been shown to suppress the development of blood vessels required for tumor growth and metastasis (Matsubara et al., 2005), and inhibit protein-activated receptor-2 inflammation that can lead to atherosclerosis (Han et al., 2003).

Furthermore, flavonoids, the largest group of plant phenolics (King and Young, 1999), are associated with lower risk of stroke (Keli et al., 1996), lung cancer (Knekt et al., 1997) and heart disease (Knekt et al., 1996). The extracts from eggplant fruit skin have demonstrated high capacity in scavenging of superoxide free radicals and inhibition of hydroxyl radical generation by chelating ferrous iron (Kaneyuki et al., 1999; Noda et al., 2000). Superoxide radicals generated *in vivo* are usually converted into hydrogen peroxide, and like other free radicals, can damage lipids, proteins, and DNA (Halliwell et al., 1995).

Nasunin, an anthocyanin isolated from the skin of purple eggplant fruit, is one phenolic compound implicated in both inhibition of hydroxyl radical generation and superoxide scavenging activity (Kaneyuki et al., 1999; Noda et al., 2000). Stommel and Whitaker (2003) distinguished at least 14 phenolic compounds among accessions in an eggplant core collection. Given the diversity of phenolic compounds in eggplants, it is

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possible that compounds other than anthocyanins contribute to high superoxide scavenging activity. Previous studies have established that extracts from purple eggplant fruit peels exhibit high capacity to scavenge both superoxide anions and hydroxyl radicals compared to most other vegetables (Kaneyuki et al., 1999). Of 120 vegetable species evaluated for antioxidant activity using four different *in vitro* antioxidant assays, eggplant was reported to rank among the top 10 species for superoxide scavenging (SOS) activity (Yang, 2006). Nasunin [delphinidin-3-(p-coumaroylrutinoside)-5-glucoside], an anthocyanin isolated from eggplants, has been shown to demonstrate high capacity to scavenge superoxide anions and inhibit generation of hydroxyl ions (Kaneyuki et al., 1999; Noda et al., 2000).

It is well documented that the quantity and quality of phenolic phytochemicals present in fruits and vegetables is significantly influenced by cultivar, environment, soil type, and growing and storage conditions (Antolovich et al., 2000; Lee et al., 2004; Naczk and Shahidi, 2004). The quality of fruit and vegetables may also be influenced by the stages of maturity and ripening. Fruit ripening is a complex developmental process that involves many specific biochemical changes in cellular metabolism and consequently, several physicochemical factors can influence the nutritional benefit of fruits. In Nigeria, both ripe and unripe eggplants (*Solanum melongena)* are often consumed either as an appetizer, desert or for leisure. It is not clear whether the ripening process could influence the antioxidant potentials of the fruits. The present study was therefore aimed at comparing the possible modulatory effect of ripening on the antioxidant potentials of eggplants.

## MATERIALS AND METHODS

### Plant material and preparation of extracts

Unripe and ripe eggplants were bought from the main market in Akure (Nigeria), and were identified at the Crop Soil and Pest Management Department of the Federal University of Technology, Akure, Nigeria. The seeds and flesh of each eggplant were separated and were designated as follows: us (unripe seed), uf (unripe flesh), rs (ripe seed) and rf (ripe flesh). Five grams each of us, uf, rs and rf were homogenized in 100 ml of distilled water, filtered using a Whatman filter paper and the filtrate were kept (at 0 - 4°C) in a refrigerator and used for analysis within a maximum of seven days. The filtrate serves as the stock solution for all determinations.

### Chemical reagents

All chemicals were purchased from Sigma (USA), Fluka Chemie (Buchs, Switzerland) and Merck (Germany).

### Experimental animals

Male adult Wistar rats (200 - 250 g) were used. The animals were used according to the standard guidelines of the Committee on Care and Use of Experimental Animal Resources.

### Determination of total phenol contents

The total phenol contents of the extracts were determined by mixing (0 - 1.0 ml) of the extracts with equal volume of water; 2.5 ml Folin-Ciocalteau's reagent and 2 ml of 7.5% sodium carbonate were subsequently added, and the absorbance was measured at 765 nm after incubating at 45°C for 40 min. The amount of phenols in the extracts was expressed as gallic acid equivalent (GAE).

### Determination of total flavonoid content

The content of flavonoids was determined using quercetin as a reference compound. Briefly, 0 - 500 µL of stock solution of extracts was mixed with 50 µL of aluminium trichloride and potassium acetate. The absorption at 415 nm was read after 30 min at room temperature. Standard quercetin solutions were prepared from 0.01 g quercetin dissolved in 20 ml of ethanol. All determinations were carried out in triplicate. The amount of flavonoids in the extracts was expressed as quercetin equivalent (QE).

### Free radical scavenging ability

The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl-2-picrylhydrazyl) free radicals were evaluated according to Gyamfi et al. (1999). Briefly, 600 µL of extracts (0 – 100 µM) was mixed with 600 µL, 0.3 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 min and the absorbance was measured at 516 nm.

### Reducing property

The reducing property was determined by assessing the ability of extracts to reduce ferric chloride ($FeCl_3$) solution as described by Pulido et al. (2000). Briefly, extracts (0 - 250 µL of stock) were mixed with 250 µL, 200 mM Sodium phosphate buffer (pH 6.6) and 250 µL of 1% potassium ferrocyanide. The mixture were incubated at 50°C for 20 min, thereafter 250 µL, 10% trichloroacetic acid was added and subsequently centrifuged at 650 rpm for 10 min. Then, 1000 µL of the supernatant was mixed with equal volume of water and 100 µL of 0.1g/100 ml ferric chloride and the absorbance was later measured at 700 nm; a higher absorbance indicates a higher reducing power.

### Iron ($Fe^{2+}$) chelating assay

The $Fe^{2+}$ chelating ability of the extracts were determined using a modified method described by Puntel et al. (2005). Freshly prepared 500 µmol/L $FeSO_4$ (150 µL) was added to a reaction mixture containing 168 µL of 0.1 mol/L Tris-HCl (pH 7.4), 218 µL saline and extracts (0 - 100µM). Then, the reaction mixture was incubated for 5 min before the addition of 13 µL of 0.25% 1,10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe (II) chelating ability was subsequently calculated with respect to the reference (which contains all the reagents without seed extract.

### Deoxyribose degradation

Deoxyribose degradation was determined by Halliwell et al. (1987). Deoxyribose is degraded by hydroxyl radicals with the release of

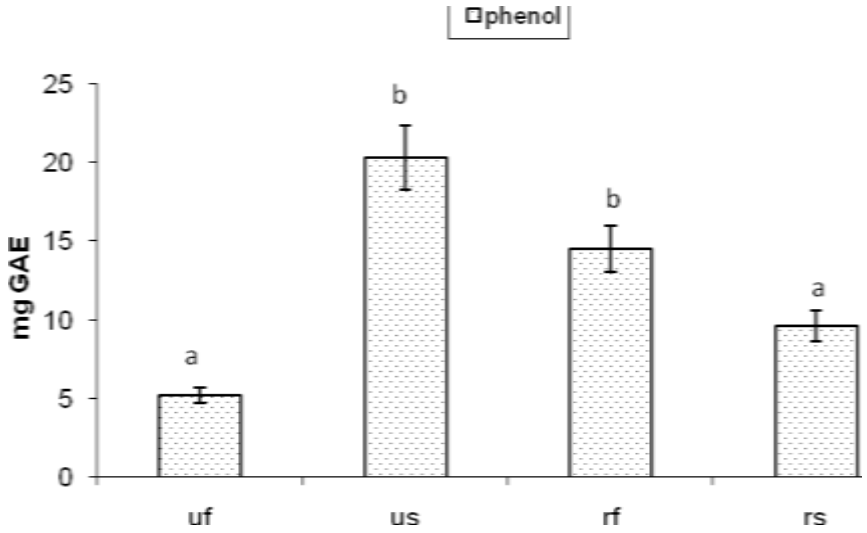


**Figure 1.** Total phenol contents of ripe and unripe eggplant. Values are given as mean ± SD of 3 to 4 independent experiment performed in triplicate and were tested by one-way ANOVA followed by Tukey's test. [b]Indicate significant difference from ([a]) at $P < 0.05$.

thiobarbituric acid (TBA) reactive materials. Deoxyribose (6 mM) was incubated at 37°C for 30 min with 50 mM potassium phosphate pH 7.4 plus $Fe^{2+}$ (0.1 mM) and/or $H_2O_2$ (1 mM) to induce deoxyribose degradation, and extracts (0 – 50 µL of stock). After incubation, 0.8 ml of 2.8% TCA and 0.4 ml of 0.8% TBA were added, and the tubes were heated for 20 min at 100°C and spectrophotometrically measured at 532 nm.

#### Lipid peroxidation

Rats were decapitated under mild ether anesthesia and the hepatic (liver) tissues were rapidly dissected, placed on ice and weighed. Tissues were immediately homogenized in cold 50 mM Tris–HCl, pH 7.4 (1/10, w/v). The homogenate was centrifuged for 10 min at 4000 g to yield a pellet that was discarded and a low-speed supernatant (S1). An aliquot of 100 ml of S1 was incubated for 1 h at 37°C in the presence of extracts, with and without the pro-oxidants, iron (final concentration (10 mM)) and sodium nitroprusside (SNP) (final concentration 3 mM). This was then used for lipid peroxidation determination. Production of thiobarbituric acid reactive species (TBARS) was determined as described by Ohkawa et al. (1979), except that the buffer of the color reaction has a pH of 3.4. The color reaction was developed by adding 300 ml 8.1% sodium dodecyl sulfate (SDS) to S1, followed by sequential addition of 500 ml acetic acid/HCl (pH 3.4) and 500 ml 0.8% thiobarbituric acid (TBA). This mixture was incubated at 95°C for 1 h. TBARS produced were measured at 532 nm and the absorbance was compared to that of the controls.

#### Statistical analysis

The results were expressed as mean ± standard error of mean (SEM) for three to four independent experiments performed in triplicate and were analyzed by one-way, two-way and three-way analysis of variance (ANOVA), followed by Turkey's test. Differences between groups were considered significant when $p<0.05$.

## RESULTS

### Phenol and flavonoid contents

The phenolic content of the seed and flesh of ripe and unripe eggplant ranged from 5.2 to 20.3 mg/g GAE. It appears that ripening diminished the phenolic content of flesh and increases the phenolic content of the seed. Conversely, ripening apparently increases the flavonoid content of both the flesh and seed of the eggplant (Figures 1 and 2).

### Free radical scavenging ability

Figure 3 shows the free radical scavenging property of the ripe and unripe eggplant. Separate one-way (within eggplant type) and two way analysis (between two eggplant types) were performed and the results indicate that all parts of the eggplants exhibits potent free radical scavenging ability. Apparently, the ripening increases the free radical scavenging potency of the flesh of eggplant and this was significant ($P < 0.05$) at a dilution of 10 fold of the stock solution.

### Reducing property

The reducing property of eggplant is presented in Figure 4. One-way analysis revealed that the reducing property of the seeds and flesh of both ripe and unripe eggplants were significant at all concentrations tested. In addition, two-way ANOVA further revealed that generally, eggplant

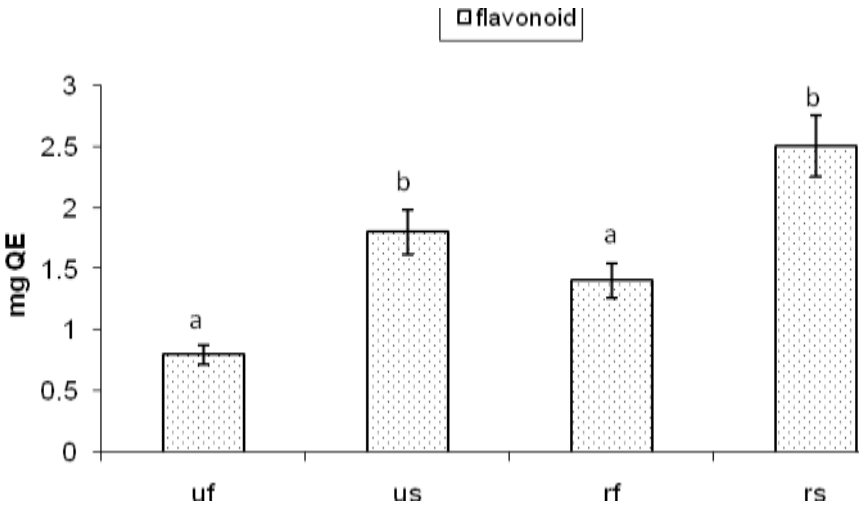


**Figure 2.** Total flavonoid content of ripe and unripe eggplant. Values are given as mean ± SD of 3 to 4 independent experiment performed in triplicate and were tested by one-way ANOVA followed by Tukey's test. [b,c,d,e]Indicate significant difference from control ([a]) at $P < 0.05$.

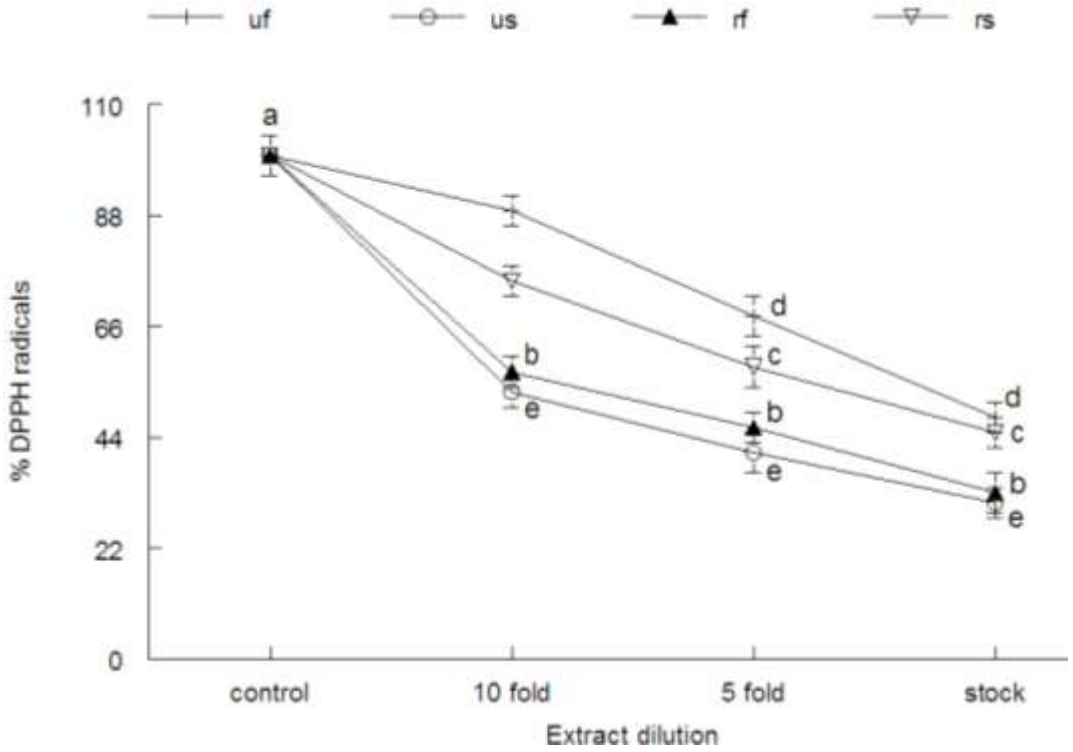


**Figure 3.** Free radical scavenging property of extracts of ripe and unripe eggplant. Values are given as mean ± SD of 3 to 4 independent experiment performed in triplicate and were tested by two-way ANOVA followed by Tukey's test. [b,c,d,e]Indicate significant difference from control ([a]) at $P < 0.05$.

is rich in free electron and readily supply such electron to $Fe^{3+}$, thereby reducing $Fe^{3+}$ to $Fe^{2+}$. However, ripening markedly increased the $Fe^{3+}$ reducing ability of both the seed and flesh of the eggplants. This reductive ability of eggplant was significant ($P < 0.05$) at the least volume of extract tested.

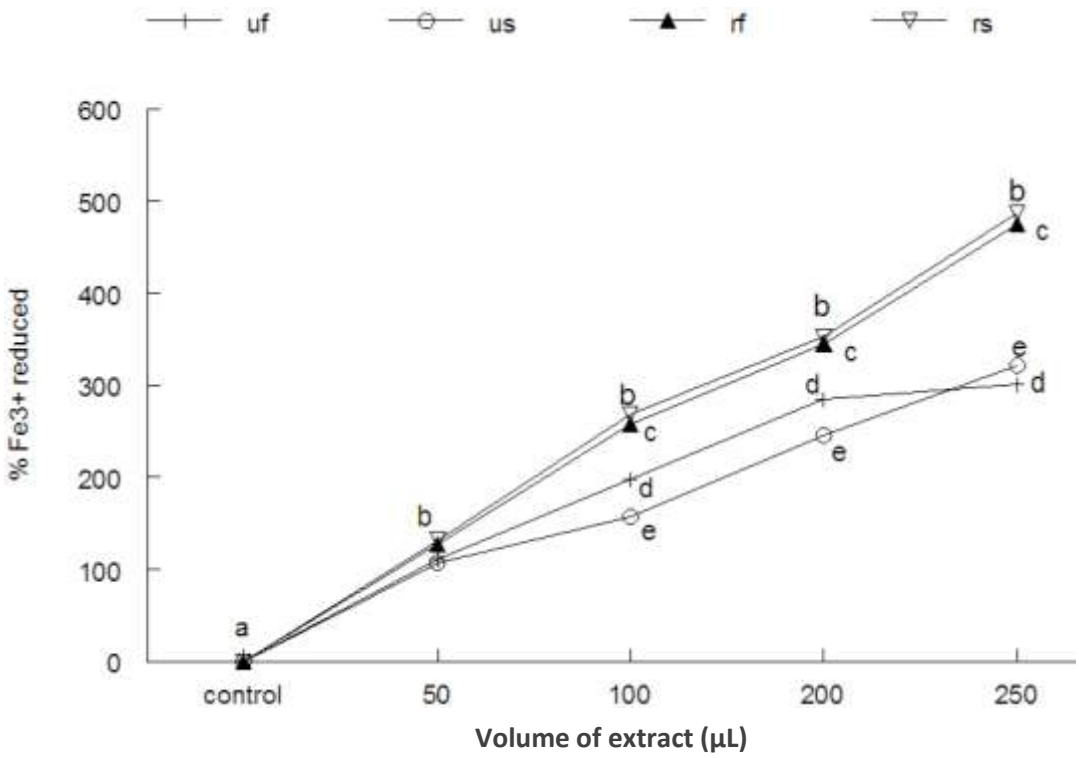


**Figure 4.** Reducing property of ripe and unripe eggplant. Values are given as mean ± SD of 3 to 4 independent experiment performed in triplicate and were tested by two-way ANOVA followed by Tukey's test. [b,c,d,e]Indicate significant difference from control ([a]) at $P < 0.05$.

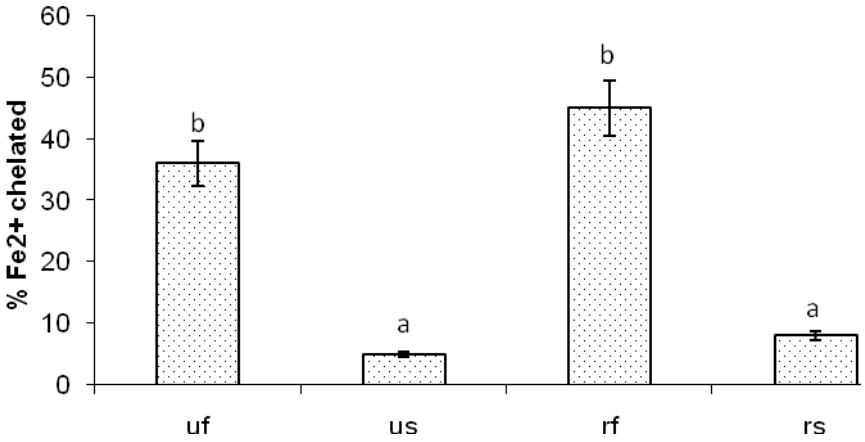


**Figure 5.** Iron chelating property of ripe and unripe eggplant. Values are given as mean ± SD of 3 to 4 independent experiment performed in triplicate and were tested by two-way ANOVA followed by Tukey's test. [b,c,d,e]Indicate significant difference from control ([a]) at $P < 0.05$.

## $Fe^{2+}$-chelating ability

Figure 5 shows the effect of ripening on the $Fe^{2+}$-chelating properties of eggplant. A two-way ANOVA followed by Tukey's test showed that the flesh of both ripe and unripe eggplant have higher chelating ability when compared to the seed. Furthermore, the chelating $Fe^{2+}$ property was markedly enhanced with ripening ($P < 0.05$). However, it is worth mentioning that one-way ANOVA showed that irrespective of the ripening stage,

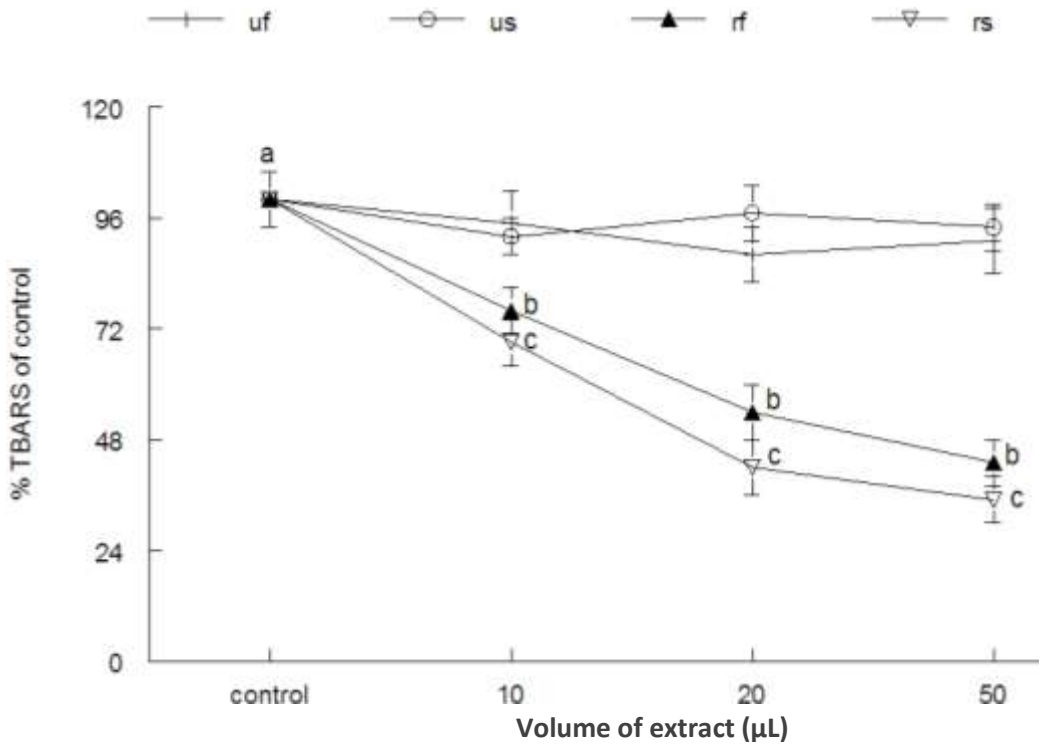


**Figure 6.** Inhibition of deoxyribose degradation by *Solanum melongena*. Values are given as mean ± SD of 3 to 4 independent experiment performed in triplicate and were tested by two-way ANOVA followed by Tukey's test. [b,c,d,e]Indicate significant difference from control ([a]) at P < 0.05.

eggplants exhibit potent iron chelating effect (p<0.05).

## Deoxyribose degradation

Figure 6 shows the inhibitory effect of seed and flesh of ripe and unripe eggplant on deoxyribose degradation under different $Fe^{2+}/H_2O_2$ oxidative assault. It is noteworthy that when $Fe^{2+}$ or $H_2O_2$ was used as the oxidants, the ripe eggplant exerted a marked inhibitory effect on deoxyribose degradation when compared to the unripe (P < 0.05). One-way ANOVA showed that individual components of the eggplants exhibited inhibitory effect on OH• radical-induced oxidative assault on deoxyribose degradation. Furthermore, three-way ANOVA showed that all parts of the eggplants exhibited potent inhibitory effect on the radical assaults on deoxyribose sugar (p<0.05)

## Lipid peroxidation

Figures 7 and 8 show the effect of ripening on the inhibitory effect of eggplant on hepatic lipid peroxidation subjected to various oxidants assaults. Figure 7 shows that when hepatic lipids are subjected to stress-induced peroxidation caused by $Fe^{2+}$ in the presence of ripe and unripe eggplant, there was a significant inhibition of the peroxidation processes. Similarly, the same pattern of results were observed when different prooxidant (sodium nitroprusside) was used in the peroxidation assay but same hepatic lipids. In fact, Figure 8 shows that when hepatic lipids were subjected to oxidative stress under sodium nitroprusside assault, both seed and flesh of eggplant were able to significantly inhibit the peroxidation of hepatic lipids in a fashion similar to that observed when $Fe^{2+}$ was used. Three-way ANOVA [4 eggplant parts × 4 concentrations of eggplants × basal/pro-oxidants] showed that the inhibitory effect of individual components of the eggplant was significant (p<0.05). Also, the two-way ANOVA revealed that irrespective of the prooxidant, the inhibitory effect of eggplant was significant at the lowest volume of extract tested (P < 0.05) and it is independent of the ripening.

## DISCUSSION

The present study demonstrated that both ripe and unripe aqueous extracts of eggplants exhibit potent antioxidant activity and apparently, the antioxidant properties of both eggplants positively correlate with their phenol and flavonoid contents. Our present finding correlates with the findings of Sudheese and his co-workers who found that

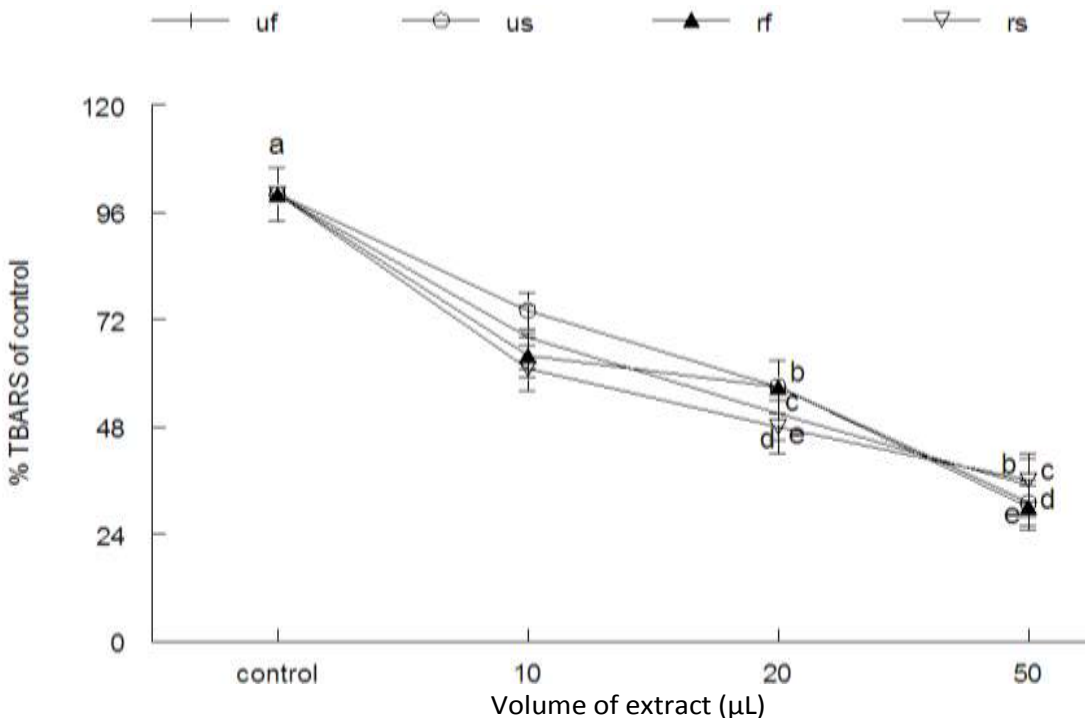


**Figure 7.** Inhibition of $Fe^{2+}$ (10 µM)-induced hepatic lipid peroxidation by ripe and unripe eggplant. Values are given as mean ± SD of 3 independent experiments performed in triplicate in different days and were tested by two-way ANOVA followed by Tukey's test. $^{b,c,d,e}$Indicate significant difference from control ($^{a}$) at $P < 0.05$.

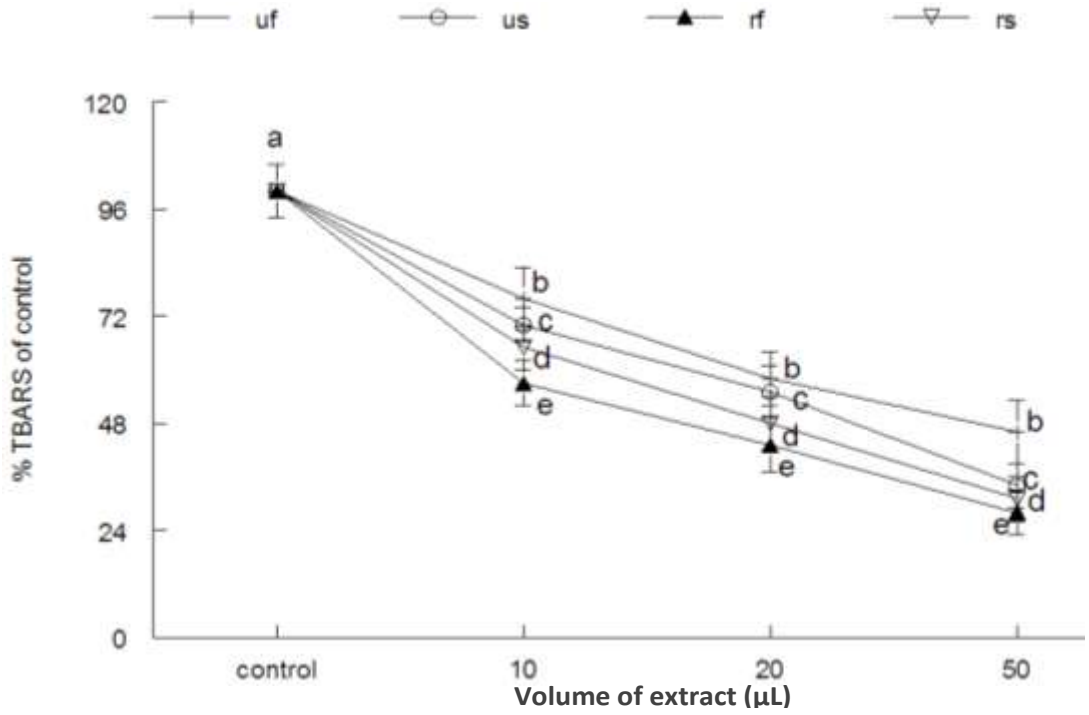


**Figure 8.** Inhibition of sodium nitroprusside (3 µM)-induced hepatic lipid peroxidation by ripe and unripe eggplant. Values are given as mean ± SD of 3 independent experiments performed in triplicate in different days and were tested by two-way ANOVA followed by Tukey's test. $^{b,c,d,e}$Indicate significant difference from control ($^{a}$) at $P < 0.05$.

flavonoids isolated from *S. melongena* showed potent antioxidant activity. In their report, they observed that rats fed 1 mg/100 g BW/day of flavonoid from the eggplants showed diminished malondialdehyde, hydroperoxides and conjugated dienes (indices of oxidative stress) in the treated animals (Sudheesh et al., 1999). In addition, the ripe fruits of eggplant may contain other compounds such as anthocyanins that give the fruits its characteristic red pigments and possibly additional antioxidant effect. In fact, extracts from purple colour small size eggplant fruits with potent antioxidant activities have been attributed to the higher phenolic and anthocyanin content (Nisha et al., 2009).

Anthocyanins are the largest group of water soluble pigments in the plant kingdom can be directly absorbed and distributed to the blood in humans and rats after consumption of the dietary anthocyanin (Bomser et al., 1999; Hagiwara et al., 2002; Matsumoto et al., 2001; Miyazawa et al., 1999). Studies related to the antioxidant capacity of anthocyanins indicate that such colorful compounds may be responsible for much of the antioxidant protection against peroxyl radicals, which cause oxidative damage to lipids, proteins, and nucleic acids, being important factors for the development of a number of diseases including cancer (Cao et al., 1996; Hou et al., 2003; Tsuda et al., 1996; Wang et al., 1997, 1999). Recent studies have shown a range of molecular evidence by which anthocyanins could act as cancer chemopreventive agents (Bomser et al., 1999; Hou et al., 2003; Kamei et al., 1995; Meiers et al., 2001). In the present study, we observed that the development of the red pigment during ripening modulates the antioxidant mechanisms of eggplants. Although Figure 3 clearly shows that both ripe and unripe flesh possess significant free radical scavenging activity, ripening exerted a potent radical scavenging activity on the eggplant and this may be related to the anthocyanin contents of the fruits. In line with free radical scavenging, the ability of eggplant to reduce $Fe^{3+}$ to $Fe^{2+}$ is also influenced by the ripening process (Figure 4). The ability of any substance to chelate and deactivate transition metals prevents such metals from participating in the initiation of lipid peroxidation, protein carbonylation, DNA assault and oxidative stress through metal-catalyzed reactions. The ability of the eggplant to chelate transition metals is therefore considered to be due to an antioxidant mechanism. The Fe(II) chelating ability of the eggplant was determined and the result is presented in Figure 5. Results indicated that the flesh demonstrated high $Fe^{2+}$ chelating property, which was positively influenced by ripening.

The oxygen molecule might produce a highly reactive oxygen species (ROS) by some exogenous factors and endogenous metabolic processes in human body. ROS include a number of chemically reactive molecules such as hydrogen peroxide ($H_2O_2$), superoxide ($O_2^-$), and the hydroxyl radical ($^{\bullet}OH$). The $^{\bullet}OH$ in the cells can easily cross cell membranes at specific sites, react with most biomolecules and furthermore cause tissue damage and cell death. Thus, removing $^{\bullet}OH$ is very important for the protection of living systems. Figure 6 shows the $^{\bullet}OH$ scavenging effect by the seeds and fruits of both the ripe and unripe eggplants extracts, and apparently the ripe eggplant extracts exhibit potent $^{\bullet}OH$ scavenging ability with consequent protection of deoxyribose damage. We may attribute this observed $^{\bullet}OH$ scavenging ability to possible anthocyanin contents that increases with ripening in the eggplant.

It also well known that iron and iron complexes stimulate lipid peroxidation in cells (Gogvadze et al., 2003). The mechanism(s) that underlies the antioxidant activity of both eggplant extracts measured in the presence of $Fe^{2+}$ may be credited to the ability of the extracts to scavenge radical, reduce $Fe^{3+}$ and chelate $Fe^{2+}$ and this would readily explain why eggplant extract exhibited marked inhibitory effect on hepatic lipid peroxidation (Figure 7). In the same vein, at physiological concentrations, eggplant extracts reduced the TBARS production induced by sodium nitroprusside (SNP) in the liver, raising the possibility of their use as possible therapeutics or preventive agents against hepatic pathologic situations. SNP has been shown to undergo photodegradation ultimately producing nitrogen oxide ($NO^{\bullet}$) $[(CN)_5\text{-}Fe]^{3+}$ and $[(CN)_4\text{-}Fe]^{2+}$ species (Arnold et al., 1984). Likewise, it has been observed that the iron moiety of SNP may have a free iron coordination site for $H_2O_2$, which could trigger the generation of highly reactive oxygen species such as hydroxyl radicals ($^{\bullet}OH$) via the Fenton reaction (Graf et al., 1984). Therefore, following a short-lasting release of NO, iron moiety of SNP could cause a long-lasting generation of $^{\bullet}OH$ radicals and oxidant stress/injury similar to that of ferrous citrate iron complexes, which may initiate a lipid peroxidation chain reaction and oxidative injury (Mohanakumar et al., 1994).

From the foregoing, we can conclude that eggplant extracts act to diminish TBARS formation in the presence of SNP by chelating $Fe^{2+}$ or reduce $Fe^{3+}$. Furthermore, since nitric oxide has been proposed to act as a pro-oxidant at high concentrations, or when it reacts with superoxide, forming the highly reactive peroxynitrite ($ONOO^{\bullet}$) (Radi et al., 2001), we can speculate that eggplant extracts may scavenge superoxide and peroxynitrite and may further interact with the cyanide moiety or even the release of iron from the ferrocyanide moiety of SNP thereby exerting a synergistic-like anti-oxidant effect. It is noteworthy to mention that although chemical measures of antioxidant activity have been employed in the present study, these measures do not represent physiological oxidation events. However, other *in vitro* studies have shown that eggplant extracts are promising candidate in the management of diabetes and hypertension (Kwon et al., 2008) and it is therefore hoped that *in vivo* data would positively correlate with the

observed *in vitro* data. In fact, available data shows that eggplants are promising agents in the management of oxidative stress-related diseases. For example, Akanitapichat et al. (2010) observed that the antioxidant activities of the eggplant were correlated with their hepatoprotective activity. In the same vein, Das and his collaborates showed that there was a direct relationship between eggplants and their cardioprotective ability (Das et al., 2011).

In conclusion, it is observed that ripening can modulate the antioxidant activity of aqueous extract of eggplant. In addition, ripening appears to induce dynamics in the polyphenol contents and consequently the antioxidative potentials between the seeds and flesh of eggplant extracts. However, since both ripe and unripe eggplants are consumed, the present data can build our confidence that the antioxidative nutritional values of eggplant are not lost when the seed and the flesh are consumed together.

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