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Effect of Cooking on Antioxidant and Enzymes Activity Linked to Carbohydrate Metabolism and Lipid Peroxidation of Eggplant (*Solanum melongena*)

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ABSTRACT

The present study is aimed at investigating the influence of cooking on antioxidant and the inhibitory ability of eggplant on enzymes linked to hyperglycemia and induced lipid peroxidation in rat's kidney (*in vitro*). The total phenolic content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity reducing power, lipid peroxidation inhibition and α-glucosidase and amylase enzymes were assayed to evaluate the effect. The results showed that cooking ($P < 0.05$) significantly increased TPC and TFC contents thereby enhancing DPPH scavenging activity and reducing power. Also α-amylase, α-glucosidase and Fe^{2+} induced lipid peroxidation inhibition in rat's kidney are enhanced significantly ($P < 0.05$) upon cooking. These results showed that cooking enhanced the ability of the eggplant to inhibit enzymes linked to diabetic mellitus and lipid peroxidation in kidney of rats *in vitro*.

Keywords: α-amylase, α-glucosidase, cooking, lipid peroxidation

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INTRODUCTION

Diabetes mellitus is a carbohydrate metabolic disorder characterized by hyperglycemia. The prevalence of diabetes mellitus has been on a steady rise in recent time and it is of concern in global public health. Not only is it difficult to treat, it has also being a major contributor to organ(s) damage, notable among them is kidney. According to National Institutes of Health (NIH), diabetes

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mellitus is one of the prominent causes of kidney damage, causing nearly twenty-five percent of kidney ailments (Afkarian et al., 2016). Studies have shown that high glucose level in the blood enhances reactive oxygen species production in mesangial cells of the kidney glomerular (Brownlee, 2005). The overproduction of these reactive oxygen species has been implicated as one of the main causes of damage to kidney (Nishikawa et al., 2000). Also, it has been well proven that oxidation of lipid is enhanced during diabetes mellitus which can lead to tissue damage (Nourooz-Zadehet al., 1997). Therefore antioxidant may be essential for the prevention and management of this diabetic complication. Hyperglycemia, an abnormal condition of excessive increase in blood level of glucose after a meal has been associated with the onset of non-insulin dependent diabetes mellitus (NIDDM) and its associated complications (Di Carli, Janises, Grunberger, & Ager, 2003). Increased postprandial blood glucose level probably occurs due to excessive activity of pancreatic enzymes; glucosidase and amylase. Evidences have been on the increase that foods possess inhibitory properties against these enzymes (Oboh et al., 2012). It is assumed that when these enzymes are inhibited, there is a significantly reduction in the postprandial elevation of blood glucose level thereby preventing hyperglycemia and hyperglycemia-induced oxidative stress. Hence it can be a therapeutic target in the management diabetic kidney disease.

Food processing such as cooking in water is usually done to improve or enhance the final qualities of food. It does not only improve flavour and taste of foods, it also enhances the bioavailability of nutrients by suppressing growth inhibitors and antinutrients in foods (Chau, Cheung, & Wong, 1997). It may also influence the total phenolics and flavonoids of food samples which may eventually affect the biological activities of such food samples (Luthria & Mukhopadhyay, 2006).

Eggplant (*Solanum melongena*) is an important agronomical plant belonging to the family Solanaceae and widely distributed throughout the temperate and tropical regions (Eun-Ju, Myung-Suk, Eun-Kyung, Young-Hong, & Seung-Cheol, 2011). It is a well consumed fruits in sub-Saharan Africa. It has many varieties across regions which usually come in different colours and shapes. It is usually eaten uncooked as snack or cooked for making stew. The eggplant is rich in polyphenol compounds and some essential vitamins (Hanson et al., 2006).

A lot of studies has shown that cooking affects the nutrients and antioxidant capacity of fruits and vegetables (Dewanto, Wu, Adom, & Liu, 2002; Chuah et al., 2008; Kao, Chiu, & Chiang, 2014). Also, many studies have reported the amylase and glucosidase inhibitory ability of eggplant extracts (Kwon, Apostolidis, & Shetty, 2008; Nwanna, Ibukun, & Oboh, 2013). The effects of processing on the physicochemical and antioxidant properties of this fruit have also been reported (Arkoub-djermoune et

al., 2016), but no study has been done to evaluate the effect of cooking on amylase, glucosidase and induced lipid peroxidation inhibitory ability of eggplant. Therefore the aim of this study is to investigate the effect of cooking eggplant on antioxidants and inhibition of enzymes linked to carbohydrate and induced lipid peroxidation in rat's kidney (*in vitro*).

MATERIALS AND METHODS

The fruits of eggplant (*Solanum melongena*) used in the work were gotten from local market (Bodija), Ibadan and identified and in the Botany Department, University of Ibadan. They were at full commercial maturity stage and eating quality. They were fresh and without infection, washed with distilled water and used for the research. The white variety usually comes in whitish yellow colouration while the green variety is usually green in colour (Figure 1).

Preparation of Cooked Sample

Fresh whole *Solanum melongena* fruit was cooked in the 500 ml-boiling water for 10 minutes. The beaker was covered to prevent water and heat loss due to evaporation. After cooking, the cooked samples were drained off. Fifty grams (50 g) of cooked sample was blended with 500 ml of distilled water using electric blender, the blended sample was filtered using muslin cloth and filtrate centrifuged at 3000 rpm (988 x *g*) for 10minutes. The supernatant was used for the chemical assays.

Preparation of Uncooked Sample

Fifty grams (50 g) of the fresh whole *Solanum melongena* fruit and was blended with 500 ml of distilled water using electric blender. The blended sample was filtered using muslin cloth and the filtrate centrifuged at 3000 rpm (988 x *g*) for 10 minutes. The supernatant was used for the chemical assays.

Figure 1. Varieties of eggplant used

Determination of Total Phenolic Content (TPC)

TPC of cooked and uncooked *Solanum melongena* was done using the (Kim, Jeong, & Lee, 2003) method with slight modifications. One millitre (1.0 ml) of the sample was mixed with 1.0 ml (10 %) of Folin-ciocalteu phenol reagent. After 5 mins, 5.0 ml (instead of 10.0) of 7 % Na_2CO_3 was added followed immediately with by addition of 5.0 ml (instead of 13.0 ml) of distilled water and shaken thoroughly. The mixture was kept in the dark for 90 minutes at room temperature. The absorbance was read at 750 nm and the TPC was evaluated from gallic acid standard curve and expressed as gallic acid equivalent (mg GAE/100g of fresh weight).

Determination of Total Flavonoid Content (TFC)

TFC of the cooked and uncooked *Solanum melongena* was done using the (Park et al., 2008) method. Sample (0.3ml) was mixed with 3.4 ml (30 %) of methanol, 0.15 ml (0.5 M) of $NaNO_2$ and 0.15 ml (0.3 M) of $AlCl_36H_20$ consecutively. After 5 minutes, 1 ml of 1 M NaOH was added and mixed well. The absorbance read at 506 nm and the flavonoid content evaluated from quercetin standard curve and expressed as quercetin equivalent (mg QUE/100g fresh weight).

Determination of 1, 1-diphenyl-2-picrylhydrazyl Scavenging Activity (DPPH)

DPPH radical scavenging activity of cooked and uncooked *Solanum melongena* was done using the (Gyamfi, Yonamine, & Aniya, 1999) method with slight modification. Appropriate dilutions of sample were added to 4 ml (instead of 3.9 ml) of DPPH solution (30 mg/l) prepared in methanol. The samples were mixed thoroughly and left in the dark for 30 minutes. The absorbance was read at 520 nm. The inhibition percentage was calculated as

Inhibition percentage of DPPH = {(Abs control- Abs Sample)/ (Abs Control)} × 100

DPPH solution without sample served as control.

Determination of Reducing Power (RP)

RP of cooked and uncooked *Solanum melongena* was done using the method of Oyaizu (1986). Appropriate dilutions of sample were mixed with 1 ml phosphate buffer (0.2 M, pH 6.6) followed by 1 ml of potassium ferricyanide (1 %) and incubated for 20 minutes at 50 ^{0}C. The reaction was terminated by 1 ml trichloroacetic acid (10 %). One millitre (1ml) of the upper portion was taken, mixed with 1 ml of distilled water and followed by 1 ml ferric chloride (0.1 %). The reaction mixture was thoroughly mixed and the absorbance was read at 700 nm. Higher absorbance indicates the higher reducing power of the sample.

Amylase Inhibition Assay (AI)

AI of the cooked and uncooked *Solanum melongena* was done using the (Worthington, 1993) method. Appropriate dilutions of sample and 500 µl (0.02 M) of sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 0.5 mg/ml α-amylase solution

were incubated at room temperature for 10 min. Thereafter, addition of 500µl (1 %) of starch solution prepared with 0.02 M sodium phosphate buffer (0.006 M sodium chloride, pH 6.9) was followed. The reaction mixtures were then incubated at room temperature for 10 min. The reaction was halted with 1.0 ml (96 mM) of dinitrosalicylic acid. The reaction mixtures were then heated in boiling water for 5 minutes and allowed to cool. The absorbance was read at 540 nm. The inhibition percentage was calculated:

Inhibition percentage = {(Abs Control-Abs Sample)/ (Abs Control)} × 100

Glucosidase Inhibition Assay (GI)

GI of the cooked and uncooked *Solanum melongena* was done using (Apostolidis, Kwon, & Shetty, 2007) method. Appropriate dilutions of sample were suspended in 1000 µl α-glucosidase solution (1.0U/L) prepared in of 0.1 M sodium phosphate buffer (pH 6.9) and incubated at room temperature for 10 min. After incubation, the reaction mixture was mixed with 500 µl (5 mM) of nitrophenyl-glucopyranoside solution prepared in 0.1 M sodium phosphate buffer (pH 6.9). The reaction mixtures were left at room temperature for 5 minutes. The absorbance was read at 405 nm and inhibition percentage calculated as follows

Inhibition percentage = {(Abs control-Abs Sample)/ (Abs Control)} × 100

Inhibition of Lipid Peroxidation (LP)

Experimental animals: - Healthy Wistar albino rats (four) of weight between 100-120 g were bought from Animal House of Physiology Department, University of Ibadan. The rats were given fed (Ladokun feed) and water *ad libitum*. They were kept under a constant 12-h light and dark cycle and acclimatized for 1-week before used for the experiment. The experimental procedures were conducted in line with procedure approved by University of Ibadan Animal Care Use and Research Ethics Committee for care and use of experimental animals.

Preparation of kidney homogenate**: -** The preparation of homogenate was done by the method of Akinyemi, Ademiluyi and Oboh (2013). The rats were sacrificed under sodium pentobarbitone anesthesia and the kidney was removed, placed on ice and weighed. Kidney was homogenized immediately in cold normal saline water (1:4 w/v of fresh weight). The kidney homogenate was centrifuged at 3000 rpm (988 x *g*) for 10 minutes. The debris was discarded and lipid-rich supernatant obtained was used for lipid peroxidation assay

LP assay**: -** LP of cooked and uncooked *Solanum melongena* was done using (Ohkawa, Ohishi, & Yagi, 1979) method of with slight modifications. The reaction mixture was made up of 200 µl of the tissue homogenate, 30 µl of 0.1M Tris-HCl buffer (pH 7.4), sample solution ((0.1 - 0.4ml) and 30 µl of the freshly prepared pro-oxidant solution (5mM Sodium nitroprusside). The reaction mixture was incubated at 37^0 C for 2 h. The chromophore was developed by adding 300 µl (8.1%) Sodium dodecyl sulphate, 600 µl (pH 3.4) of acetic acid and

600 µl (0.8%) of TBA consecutively to the reaction mixture, thereafter the reaction mixtures were incubated for 1 hour at 100 ^{0}C. The absorbance of TBA-adduct (Malondialdehyde) formed was monitored at 532 nm. Inhibition of Malondialdehyde (MDA) production was calculated by the method of Banerjee (2005).

Statistical Analysis

Data are expressed as the mean ± SD of three measurements. The significance of the differences between the means of the samples were established by the analysis of variance using (SPSS 20) least significant difference $P< 0.05$, charts were drawn with graph pad prism 5 and Pearson correlation test was conducted to determine the correlation between antioxidant activities and enzymes inhibition. Significant levels were established using $P< 0.05$.

RESULTS

The effect of cooking on TPC and TFC of *Solanum melongena* was showed in Table 1. The TPC of uncooked white and green varieties of *Solanum melongena* were 72.50 mgGAE/100gfw and 70.2 mg GAE/100gfw respectively while TFC were 37.00 mgQUE/100gfw and 42.50 mgQUE/100gfw for white and green varieties respectively. On cooking, the total phenolics and total flavonoids increased significantly. TPC increased to 86.80 mg GAE/100gfw (white) and 86.20 mg QUE/100gfw (green) while TFC increased to 129.00 mg QUE/100gfw (white) and 177.00 mg QUE/100gfw (green). Cooking also significantly enhanced the capacity of the *Solanum melongena* to scavenge DPPH to Diphenylpicrylhydrazine with the loss of its violet colour (Figure 2). The uncooked samples were found to have an IC_{50} of 0.940 ml and 0.854 ml for white and green varieties respectively. Upon cooking the IC_{50} were found to decrease 0.386 ml and 0.321 ml respectively. Also reducing ability of the *Solanum melongena* was significantly enhanced in both varieties upon cooking in water (Figure 3).

The lipid peroxidation assay showed that the capacity of the *Solanum melongena* samples to inhibit the production of MDA in rat's kidney (*in vitro*) increased (Figure 4). The uncooked samples had the maximum inhibition of 63.34 % (white) and 63.70 % (green). Upon cooking, the inhibition increased to 78.25 % (white) and 82.84% (green). The increase in percentage inhibition of MDA produced was statistically significantly between the cooked and uncooked *Solanum melongena* samples. Also similar trend was observed in α-glucosidase and α-amylase inhibition assays. For α-glucosidase and α-amylase inhibitions (Figures 5 and 6), the uncooked *Solanum melongena* had maximum inhibition of 36.35% (white) and 37.85% (green), upon cooking, it significantly increased to 40.2% (white) and 45.85% (green). Cooking also significantly increased inhibition of α-glucosidase, the uncooked *Solanum melongena* had maximum inhibition of 22.71% (white) and 25.04% (green), while the cooked *Solanum melongena* had 28.24 % (white) and 31.53% (green) (Tables 2 and 3).

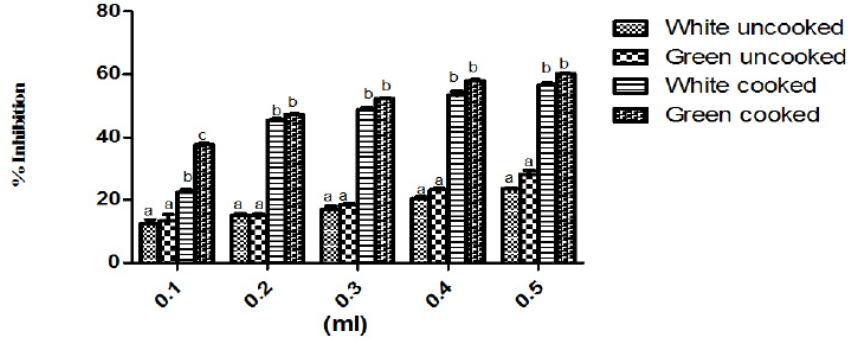

Figure 2. DPPH scavenging activity of cooked and uncooked *Solanum melongena*. Values were presented as Mean ± SD of triplicate reading. Values with the same superscript letter on grouped bars are not different significantly ($P < 0.05$)

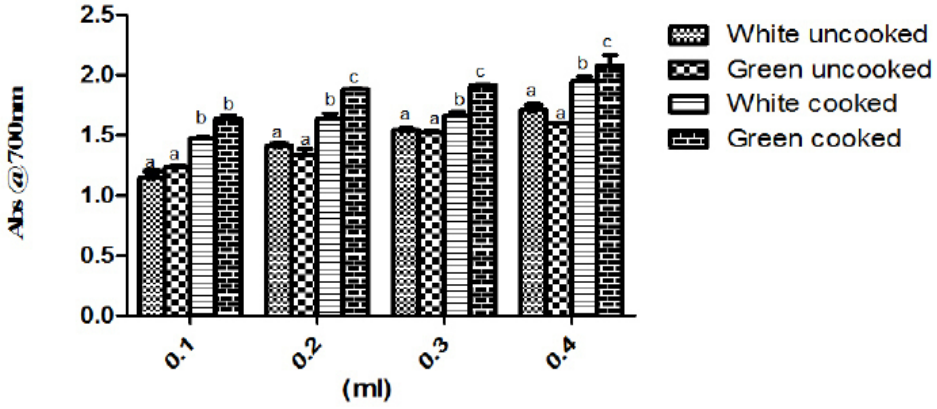

Figure 3. Reducing ability of cooked and uncooked *Solanum melongena*. Values were presented as Mean ± SD of triplicate reading. Values with the same superscript letter on grouped bars are not different significantly ($P < 0.05$)

Values followed by similar letters under the same column are not significantly different at p = 0.05 according to Duncan's multiple range test.

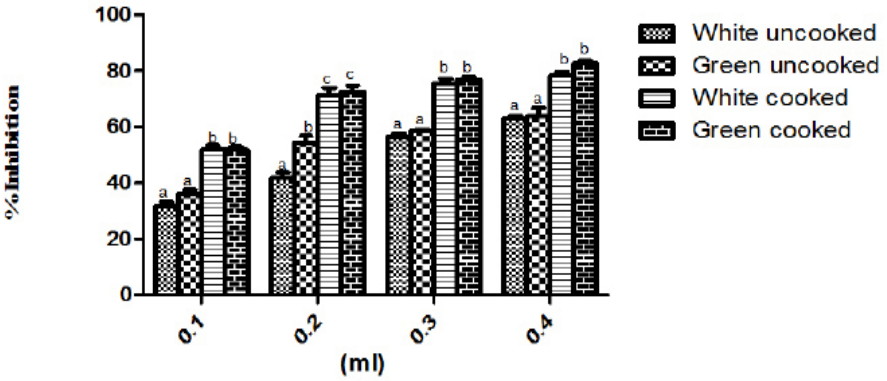

Figure 4. Inhibition of Fe^{2+} induced MDA production in Kidney by cooked and uncooked *Solanum melongena*. Values were presented as Mean ± SD of triplicate reading. Values with the same superscript letter on grouped bars are not different significantly ($P < 0.05$)

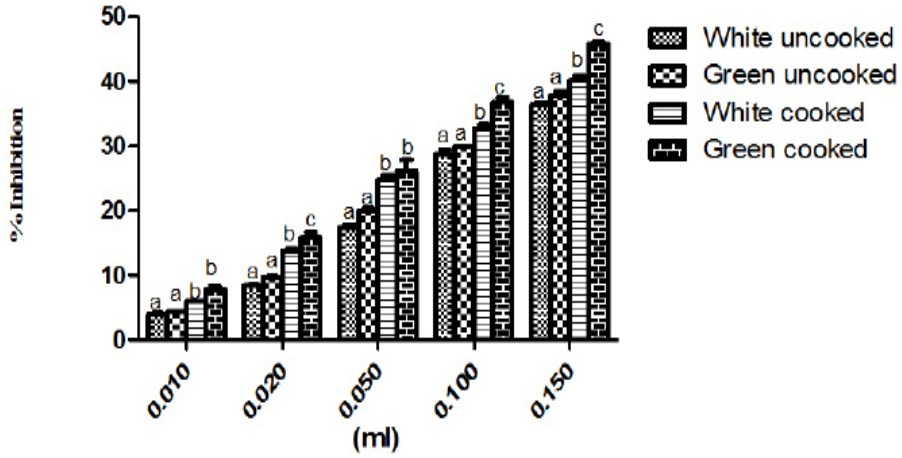

Figure 5.α-glucosidase inhibition ability of cooked and uncooked *Solanum melongena*. Values were presented as Mean ± SD of triplicate reading. Values with the same superscript letter on grouped bars are not different significantly ($P < 0.05$)

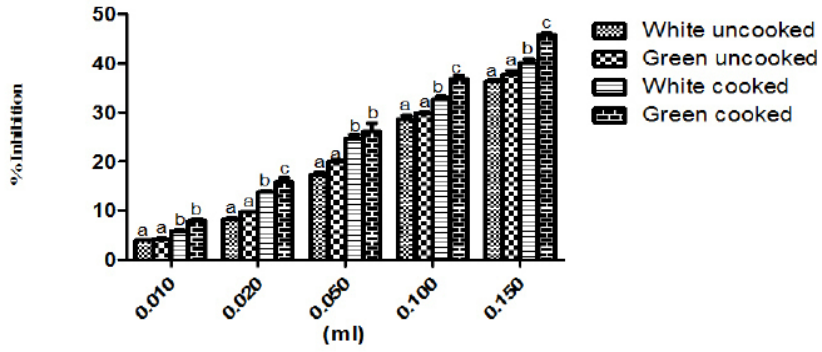

Figure 6.α-amylase inhibition ability of cooked and uncooked *Solanum melongena*. Values were presented as Mean ± SD of triplicate reading. Values with the same superscript letters on grouped bars are not different significantly ($P < 0.05$)

Table 1

Effect of cooking on TPC and TFC of cooked and uncooked Solanum melongena

	WU	GU	WC	GC
TPC	72.50±0.06^{a}	70.20±0.05^{a}	86.80±0.04^{b}	86.20±0.04^{b}
TFC	37.00±0.02^{a}	42.50±0.08^{b}	129.00±0.04^{c}	177.00±0.06^{d}

TPC: Total phenolic content (mg GAE/100 g fw), TFC; Total flavonoid content (mg QUE/100 g fw). WU; white uncooked, GU; Green uncooked, WC; White cooked, GC; Green cooked. Values were presented as Mean

Table 2

IC_{50} (ml) of uncooked and cooked Solanum melongena *on DPPH, α-amylase, α-glucosidase and MDA production*

	WU	GU	WC	GC
DPPH scavenging	0.94±0.06^{c}	0.85±0.04^{c}	0.39±0.03^{b}	0.32±0.03^{a}
α-amylase activity	0.49±0.04^{c}	0.41± 0.05^{b}	0.32±0.05^{a}	0.29±0.04^{a}
α-glucosidase activity	0.43±0.04^{c}	0.40±0.03^{b}	0.37±0.03^{b}	0.21±0.02^{a}
MDA	0.28±0.02^{d}	0.19±0.02^{c}	0.16±0.01^{b}	0.10±0.01^{a}

WU; white uncooked, GU; Green uncooked, WC; White cooked, GC; Green cooked. Values were presented as Mean ± SD of triplicate reading. Values with similar letter across the row are not different significantly ($P < 0.05$).

Table 3

Correlations between DPPH scavenging activity and inhibition of enzymes of carbohydrates metabolism

	Antioxidant	Glucosidase	Amylase
Antioxidant	1		
Glucosidase	0.59*	1	
Amylase	0.57*	0.98*	1

∗Correlation is significant at 0.05 level ($P < 0.05$).

DISCUSSION

Numerous studies have shown that there is positive correlation between sound health and consumption of foods rich in polyphenolic phytochemicals, also cooking or thermal treatments have been shown to alter the polyphenol contents, antioxidants and health functionality of fruits and vegetables (Chuah et al., 2008; Randhir, Kwon, & Shetty, 2008). Therefore, this study was designed to ascertain the effect of water cooking on antioxidant and inhibitory ability of *Solanum melongena* on enzymes linked to hyperglycemia and Fe^{2+} induced lipid peroxidation in kidney of rats (*in vitro*).

Numerous studies have shown conflicting results on effect of cooking in water on the polyphenolic contents, antioxidant ability and health functionality of fruits and vegetables. Although some water soluble or high polarity antioxidants could have been leaked into the water medium but the result obtained in this study showed that cooking in water enhanced the polyphenolic contents, antioxidant and inhibitory ability of eggplant on enzymes linked to hyperglycemia and Fe^{2+} induced lipid peroxidation in kidney of rats (*in vitro*). Polyphenolic are regarded as the major contributors of *in vitro* antioxidant capacity in foods (Li, Pickard, & Beta, 2007). In this study cooking in water enhanced the polyphenolic content of the eggplants used. Hence, the enhanced polyphenolic contents could be due to the mobilization of bound polyphenolic from the breakdown of cellular components and cell walls, other reasons could be that phenolics other than the endogenous ones may be formed as by-products during heat treatment in the eggplants (Randhir et al., 2008), higher extractability of antioxidant components due to matrix softening by cooking (Bernhardt & Schlich, 2006) and liberation of active aglycones from flavonoid conjugates (Miglio, Chiavaro, Visconti, Fogliano, & Pellegrini, 2008; Podsedek, 2007). This result is in agreement with the study of Dewanto et al. (2002) and Turkmen, Sari, &Velioglu (2005) who reported enhanced antioxidant activity in fruits and vegetables upon cooking treatments. Though our study did not extend to chemical profiling of eggplants but previous study by Lo-Scalzo et al. (2010) had reported that cooked eggplant were much richer in phenolic

compounds like caffeic and chlorogenic acids when compared with raw eggplants.

The lipid peroxidation process is induced by the pro-oxidant effect of transition metals. This transition metal mediates the production of lipid peroxides by stimulation of the oxidative machinery ($OH^{\cdot}$) through haber-weiss reaction but the rate constant of this reaction *invivo* is lower than that of the dismutation reaction and would not proceed significantly. However this method has been successfully used to investigate *in vitro* oxidation of lipid since Fenton reported that a mixture of hydrogen peroxide and ferrous salts was an effective oxidant agent (Repetto & Boveris, 2012). Apart from the fact that this procedure can be induced *in vitro*, it has high precision and accuracy. In this study, our results showed that the ability of both varieties of eggplant to inhibit Fe^{2+} induced lipid peroxidation in rat's kidney (*in vitro*) was increased upon cooking in water. This increased inhibition can be attributed to enhanced polyphenolics and antioxidant potential of eggplant upon cooking since several studies have shown that lipid peroxidation can be averted or reduced by antioxidant compounds. Our results showed similar trend with the study of Lo-Scalzo et al. (2010) where cooking enhanced the ability of eggplant to inhibit oxidative burst in human neutrophil (*in vitro*) via enhancement of phenolic compounds. Previous *in vitro* results from Nwanna et al. (2013) and Kwon et al. (2008) have shown that eggplants possess amylase and glucosidase inhibitory activity. In this study, the ability of eggplant to inhibit α-amylase and α-glucosidase enzymes was increased significantly upon treatment by cooking in water. It could be due to the phenolic oxidation, polymerization or changes in phenolic profile due to thermal processing (Randhir et al., 2008). Also our results showed positive correlation between the increased antioxidant capacity and enzymes inhibition which are also in agreement with the study of Randhir et al. (2008) where thermal treatment increased α-amylase inhibition activity in oat and buckwheat grains and increased α-glucosidase inhibition activity in wheat, buckwheat and oats.

CONCLUSION

In conclusion, our findings in this study have shown that the ability of eggplant to inhibit α-amylase, α-glucosidase and Fe^{2+} induced lipid peroxidation in rat's kidney (*in vitro*) is significantly ($P < 0.05$) enhanced by water cooking. This can be attributed to the ability of water cooking to increase bioaccessibility of polyphenol in eggplant.

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