Effect of cooking on the antioxidant properties of two varieties of bitter yam (*Dioscorea dumetorum*)

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ABSTRACT: This study sought to evaluate the effect of cooking on antioxidant activity and protective ability of water extractable phytochemicals from two varieties of *Dioscorea dumetorum* [Bitter yam (white and yellow)] against lipid peroxidation induced by FeSO$_4$ in rat brain in vitro. The results of the study revealed that incubating brain tissue in the presence of 25μM FeSO$_4$ caused a significant increase (p<0.05) in thiobarbituric reactive species (TBARS) produced in the rat’s brain (134%) when compared with the basal (100%). In addition, bitter white yam (raw and cooked) had a significantly (p<0.05) higher total phenol, flavonoid, DPPH*, ABTS* and Fe$^{3+}$ chelating ability than bitter yellow yam (raw and cooked). It is therefore concluded that bitter yams in general prevent Fe$^{2+}$ induced lipid peroxidation, with bitter white yam (raw and cooked) being more potent. Meanwhile cooked bitter white yam had the highest protective ability. This is probably due to its higher phenolic contents (phenol and flavonoid), Fe$^{3+}$ chelating, ferric reducing property and DPPH* free radical scavenging ability.

**Key words:** Bitter yam, antioxidant, raw, cooked, *Dioscorea dumetorum*

INTRODUCTION

Foodstuffs supply not only energy, essential amino acids, fiber, vitamins, and minerals but also some active compounds such as antioxidants (tocopherols, carotenoids, vitamin C, phenolic compounds, etc.) that may have different beneficial functions in the body. Dietary components, which are capable of acting as antioxidants, are likely to be beneficial by augmenting cellular defenses and protecting the cell against damage caused by free radicals, by acting as radical scavengers, reducing agents, potential complexes of prooxidant metals, and quenchers of singlet oxygen formation (Doblado et al., 2005; Gutteridge, 1993; Hochstein and Atallah, 1988; Oboh, 2005; Oboh, 2006). *Dioscorea dumetorum* is an important food security crop of all the yam species mostly consumed in west Africa. *D. dumetorum* has pale to deep yellow pigmentation. It is consumed primarily in Ghana, Nigeria, Cameroon, Guinea and Mali. However, *D. dumetorum* is not consumed as much as the two highest consumed yam species, *D. rotundata* (white yam) or *D. alata* (water yam). *D. dumetorum* is generally prepared by prolonged soaking and boiling and has a mild bitter taste that is attractive to some but not to others and undergoes tuber hardening during storage (Afoakwa and Sefa-Dedeh, 2001). However, *D. dumetorum* has been reported to be the most nutritious of the six yam species consumed (Agbor-Eghe and Teche, 1995). It is reported to have a mean protein content of 9.6% (dry matter basis) compared to 8.2% for *D. alata* and 7.0% for *D. rotundata*. Also it has been

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shown to have a higher nutritional quality than white yam for growing rats (Agbor-Egbe and Teche, 1995). Although Fe is necessary in relatively large amounts for hemoglobin, myoglobin and cytochrome production, xanthine oxidase and the other Fe proteins require rather small amounts of Fe. On the other hand, free Fe in the cytosol and in the mitochondria can cause considerable oxidative damage by increasing superoxide production, through Fenton reactions and by activating xanthine oxidase, which produces both uric acid (an antioxidant that recycles ascorbic acid in the cell and is therefore vital to the animals that do not produce ascorbic acid, such as primates) and $O_2^-$, which causes massive damage either by itself or by reacting with nitric oxide (NO) to form the powerful peroxynitrite (ONOO') (Johnson, 2001). This study was designed to investigate the effects of processing on the antioxidant properties of two varieties of Dioscorea dumetorum.

**MATERIALS AND METHODS**

Bitter yam (white and yellow) tubers were sourced locally from the Oba’s market in Akure, Ondo State, Nigeria. The identification and authentication was done at the Crop, Soil, and Pest management (CSP) Department of the Federal University of Technology, Akure, Nigeria. All the chemicals used were of analytical grade, while the water was glass distilled.

**Sample preparation**

The bitter yam (white and yellow) samples were washed, peeled and sliced into about 5.0 cm diameter slices with 1.0 cm thickness and dried. The sun dried samples were ground to powder and kept dry before analysis, some portion of the yams were boiled and later sun dried and ground to powder for further analysis.

**Aqueous extract preparation**

Briefly, 1g of the powdered samples (Raw and Cooked) were soaked in 20ml of distilled water overnight, then centrifuged at 3000rpm for 10min. The supernatant collected was then kept at about 4°C for further analysis.

**Determination of total phenol content**

The total phenol content of the extracts was determined using the method reported by Singleton et al. (1999). 0.5mL of the extracts were oxidized with 2.5mL of 10% Folin–Ciocalteau’s reagent (v/v) and neutralized by 2.0mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance was measured at 765 nm. The total phenol content was subsequently calculated using Gallic acid as standard.

**Determination of total flavonoid content**

The total flavonoid content of the extracts was determined using a slightly modified method reported by Meda et al. (2005). Briefly, 0.5mL of the sample was mixed with 0.5 mL methanol, 50μL of 10% A KCl, 50μL of 1mL L⁻¹ potassium acetate and 1.4mL water, and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was subsequently measured at 415 nm. The total flavonoid was calculated using Quercetin as standard.

**DPPH free radical scavenging ability**

The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl-2-picyrylhydrazyl) free radical was evaluated as described by Gyamfi et al. (1999). Briefly, an appropriate dilution of the extracts (1mL) was mixed with 1mL
of 0.4 mmol L⁻¹ methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min and the absorbance was measured at 516 nm. The DPPH free radical scavenging ability was subsequently calculated with respect to the reference (which contains all the reagents without the test sample).

**2,2-azinobis(3-ethylbenzo-thiazoline-6-sulfonate) ABTS**’ scavenging ability

The ABTS’ scavenging ability of the extracts was determined according to the method described by Re et al. (1999). ABTS’ was generated by reacting an ABTS aqueous solution (7 mmol L⁻¹) with K₂S₂O₅ (2.45 mmol L⁻¹, final concentration) in the dark for 16 h and adjusting the Abs 734nm to 0.700 with water. 0.2mL of appropriate dilution of the extract was added to 2.0mL ABTS’ solution and the absorbance were measured at 734nm after 15 min. The antioxidant capacity was subsequently calculated using trolox as standard.

**Ferric reducing antioxidant property**

The reducing property of the extracts was determined by assessing the ability of the extract to reduce FeCl₃ solution as described by Oyaizu (1986). A 2.5mL aliquot was mixed with 2.5mL of 200mmol L⁻¹ sodium phosphate buffer (pH 6.6) and 2.5mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and then 2.5mL of 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. 5mL of the supernatant was mixed with an equal volume of water and 1mL of 0.1% ferric chloride. The absorbance was measured at 700 nm and ferric reducing antioxidant property was subsequently calculated using ascorbic acid as standard.

**OH Radical Scavenging Ability**

The ability of the extract of the yam extract to prevent Fe²⁺/H₂O₂-induced decomposition of deoxyribose was carried out using the method of Halliwell and Gutteridge, 1981. Briefly, freshly prepared aqueous extract was added to a reaction mixture containing 120 [20 mM] deoxyribose, 400 [0.1 M] phosphate buffer, 40 [20 mM] hydrogen peroxide, and 40 [500 mM] FeSO₄, and the volume were made up to 800 mL with distilled water. The reaction mixture was incubated at 37°C for 30 min and the reaction was stopped by the addition of 0.5 mL of 2.8% trichloroacetic acid; this was followed by the addition of 0.4 mL of 0.6% thiobarbituric acid (TBA) solution. The tubes were subsequently incubated in boiling water for 20 min. The absorbance was measured at 532 nm in a spectrophotometer.

**Fe²⁺ chelation assay**

The Fe²⁺ chelating ability of the extracts were determined using a modified method of Minotti and Aust (1987) with a slight modification by Puntel et al. (2005). Freshly prepared 500 [1 mL] FeSO₄ (150 mL) was added to a reaction mixture containing 168 [0.1 mol L⁻¹] Tris-HCl (pH 7.4), 218 [saline and the extracts]. The reaction mixture was incubated for 5 min, before the addition of 13 [0.25% 1,10-phenanthroline (w/v)]. The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe²⁺ chelating ability was subsequently calculated.

**Lipid peroxidation**

**Tissue preparation:** The brain of the rat was quickly removed, placed on ice and weighed. This tissue was subsequently homogenized in cold saline (150 mM NaCl solution) Tissue: saline (1:10 w/v) using mortar and pestle. The homogenate was centrifuged for 10 minutes at about 2000 rpm to yield a pellet that was discarded and a low-speed supernatant (SI) was kept for lipid peroxidation assay (Puntel et al., 2005).
Lipid peroxidation and Thiobarbituric acid reactions:
The lipid peroxidation assay was carried out according to the method of (Ohkawa et al., 1979) as modified by (Puntel et al., 2005) using Iron (II) sulphate as a prooxidant. Briefly, 1 mL of the SI fraction was mixed with a reaction mixture containing 0.30 mL 10 mM Tris-HCl (pH 7.4), and the volume was made up to 3 mL by with distilled water then 0.3 mL of the extract was added before incubation at 37°C for 1 hr. The colour reaction was developed adding 3 mL of 8.1 % SDS (Sodium dodecyl sulphate) to the reaction mixture containing SI, this was subsequently followed by the addition of 5 mL acetic acid solution pH 3.4) and 5 mL 0.6 % TBA (thiobarbituric acid). This mixture was then incubated at 100°C for 1 hr. Thiobarbituric acid reactive species (TBARS) produced were measured at 534 nm and the concentration was extrapolated from the standard curve of malondialdehyde (MDA).

Data analysis
The results of replicate readings were pooled and expressed as mean ± standard deviation. One way analysis of variance was used to analyze the results and Duncan multiple test was used for the post hoc (Zar, 1984).

RESULTS AND DISCUSSION
The total phenol distribution in the aqueous extract of the two varieties of bitter yam [Dioscorea dumetorum (white and yellow)] is presented in Table 1. The result of total phenol ranges from 2.18 - 2.42 mg/g showing bitter white yam raw having the highest phenol content while bitter yellow yam raw has the least. However, the total phenol content of both yam varieties was higher than that reported for wild yam (Bhandari and Kawahata, 2004); brown yam (Farombi et al., 2000) and yam flour and paste (Adedayo et al., 2012). Phenolics form a wide group of natural antioxidants present in a large number of plant foods. They contribute to food characteristics such as taste, colour or shelf-life. They also participate in the prevention of several major chronic diseases such as cardiovascular diseases, diabetes, cancers or neurodegenerative diseases (Neveu et al., 2010) whose pathogenesis have been linked to the action of free radicals.

The result of the total flavonoid content distribution in the aqueous extract of the two varieties of bitter yam [Dioscorea dumetorum

Table 1: Total phenol, total flavonoid, ABTS and reducing power of two varieties of bitter yams (white and yellow)

<table>
<thead>
<tr>
<th></th>
<th>Raw white yam</th>
<th>Cooked raw</th>
<th>Raw yellow yam</th>
<th>Cooked yellow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenol (mgGA/g)</td>
<td>2.42 ± 0.00</td>
<td>2.38 ± 0.00</td>
<td>2.18 ± 0.01</td>
<td>2.24 ± 0.03</td>
</tr>
<tr>
<td>Total flavonoid (mg/g)</td>
<td>0.47 ± 0.07</td>
<td>0.68 ± 0.05</td>
<td>0.11 ± 0.01</td>
<td>0.37 ± 0.03</td>
</tr>
<tr>
<td>ABTS</td>
<td>1.59 ± 0.00</td>
<td>1.55 ± 0.00</td>
<td>1.52 ± 0.00</td>
<td>1.56 ± 0.00</td>
</tr>
<tr>
<td>Scavenging ability</td>
<td></td>
<td></td>
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<tr>
<td>(mmol TEAC/100g)</td>
<td></td>
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<tr>
<td>Reducing power</td>
<td>0.19 ± 0.01</td>
<td>0.40 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td>0.41 ± 0.01</td>
</tr>
<tr>
<td>(mg AAE/g)</td>
<td></td>
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</table>

Values represent mean ± standard deviation of replicate readings. Values with same letter along the same column are not significantly different (P < 0.05). GAE: gallic acid equivalent; QE: quercetin equivalent; TAEC: Trolox equivalent antioxidant capacity; AAE ascorbic acid equivalent.
(white and yellow)) is presented in Table 1. The result of the total flavonoid content ranges from 0.11 - 0.68 mg/g with bitter white yam cooked having the highest value while bitter yellow yam raw has the lowest. Cooking caused a significant (P < 0.05) increase in the total flavonoid contents of both cultivars. Flavonoids are polyphenolic compounds known for their high antioxidant properties and free radical scavenging ability (Scherer and Godoy, 2009). Studies have revealed that consumption of flavonoid-rich foods and plants could help in the management of hypertension (Kwon et al., 2006). They may also prevent the progressive impairment of pancreatic cells function due to oxidative stress and thus reduce the occurrence of type-2 diabetes (Song et al., 2005).

Reducing power is an antioxidation defense mechanism that involves the electron and hydrogen atom transfer strength of antioxidant molecules (Dastmalchi et al., 2007). Ferric-to-ferrous iron reduction occurs rapidly with all reductants with half reaction reduction potentials above that of Fe³⁺/Fe²⁺, the values in the ferric reducing power assay will express the corresponding concentration of electron-
The ferric reducing antioxidant power (FRAP) of the aqueous extract of the two varieties of bitter yam [Dioscorea dumetorum (white and yellow)] is also presented in Table 1 as ascorbic acid equivalents (AAE). The result revealed that bitter yellow yam cooked had the highest reducing power (0.41mg/g AAE) while bitter white yam raw had the least (0.19mg/g AAE). Also, cooking caused significant (P<0.05) increase in the reducing abilities of both yam cultivars (white and yellow).

The Fe2⁺ chelating ability of the aqueous extracts of the two varieties of bitter yam [Dioscorea dumetorum (white and yellow)] is presented in Figure 1. The result revealed that the extracts of the two yam varieties were able to chelate Fe2⁺ at the concentration of the extract tested (2.17 mg/ml); however, aqueous extract of bitter white yam cooked had the highest Fe2⁺ chelating ability while the aqueous extract of bitter yellow yam raw had the least. Cooking on the other hand caused a significant (P < 0.05) increase in the iron chelating ability of the yam varieties. The mechanism by which iron can cause its deleterious effect is that Fe2⁺ can react with hydrogen peroxide (H₂O₂) to produce hydroxyl radical (OH⁻) via the Fenton reaction, whereas superoxide can react with Fe2⁺ to regenerate Fe3⁺ that can again participate in the Fenton reaction (Fraga and Oteiza, 2002). Hence, potent iron chelators are desirable in preventing iron from initiating free radical generation via the Fenton reaction.

The hydroxyl radical (OH⁻) radical scavenging abilities of the aqueous extracts of the two yam varieties (white and yellow) is presented in Figure 2. The results revealed that both extracts of the two yam varieties were able to scavenge OH⁻ produced from the decomposition of deoxyribose in Fenton reaction at the concentration of the extract tested (0.74 mg/ml). However, bitter white yam raw had the highest OH⁻ radical scavenging ability while bitter white yam cooked had the least at the concentration of the extract tested. However, the raw extract of both varieties are more potent than the cooked samples.

The antiradical activity of phenolics is majorly based on the redox properties of their hydroxyl

![Figure 3: DPPH radical scavenging ability of extracts of two varieties of bitter yams (White and Yellow)](image)

Key:  
BWR - Bitter white yam raw  
BWC - Bitter white yam cooked  
BYR - Bitter yellow yam raw  
BYC - Bitter yellow yam cooked
Antioxidant properties of two varieties of bitter yam (Dioscorea dumetorum)

groups and the structural relationships between different parts of their chemical structure (Rice-Evans et al., 1996). The DPPH free radical scavenging ability of the aqueous extract of the two varieties of bitter yam [Dioscorea dumetorum (white and yellow)] is presented in Figure 3. The results revealed that both aqueous extracts of the yams scavenged DPPH radicals in a concentration-dependent pattern (0 – 10 mg/ml). However, bitter white yam cooked has the highest DPPH free radical scavenging ability while bitter yellow yam cooked had the least. Cooking improved the free radical scavenging ability of the white cultivar of the bitter yam and not that of the yellow cultivar. DPPH is frequently used in the determination of free radical scavenging ability; however, it has the limitation of color interference and sample solubility. Therefore, the free radical scavenging ability of the yam extracts was further studied using a moderately stable nitrogen-centered radical species: ABTS*. The ABTS* radical-based model of free radical scavenging ability has the advantage of being more versatile as both nonpolar and polar samples can be assessed and spectral interference is minimized as the absorption maximum used is 760 nm, a wavelength not normally encountered with natural products (Re et al. 1999). The ABTS’ free radical scavenging ability of the aqueous extract of the two varieties of bitter yam [Dioscorea dumetorum (white and yellow)] is presented in Table 1 as trolox equivalent antioxidant capacity (TEAC). The result revealed that bitter white yam raw (1.59 mmol/100g) had the highest ABTS’ scavenging ability while bitter yellow yam raw (1.52 mmol/100g) had the least. The result of the ABTS’ free radical scavenging ability of the aqueous extract of the two varieties of bitter yam revealed that cooking caused a slight decrease in the scavenging ability of the white cultivar while cooking caused a slight increase in the yellow cultivar. Lipid peroxidation is an autocatalytic process, which is a common consequence of cell death. This process may cause peroxidative tissue damage in inflammation, cancer and toxicity of xenobiotics and aging. Malondialdehyde (MDA)

![Graph showing inhibition of Fe (II) induced lipid peroxidation in rat brain by aqueous extracts of two varieties of bitter yam (white and yellow)](image)

Figure 4: Inhibition of Fe (II) induced lipid peroxidation in rat brain by aqueous extracts of two varieties of bitter yam (white and yellow)

Key: BWR - Bitter white yam raw  
BWC - Bitter white yam cooked  
BYR - Bitter yellow yam raw  
BYC - Bitter yellow yam cooked
is one of the end products in the lipid peroxidation process (Kurata et al., 1993). The ability of the two varieties of bitter yam [Dioscorea dumetorum (white and yellow)] to inhibit Fe$^{3+}$ induced lipid peroxidation in rat’s brain (in vitro) is presented in Figure 4. The incubation of rat’s brain with Fe$^{3+}$ caused a significant increase in the thiobarbituric reactive substances (TBARS) formation in the brain homogenate (134.1%); however, all the extracts caused significant decrease in the TBARS production in the rat’s brain in a concentration-dependent manner (0 – 160µg/ml). Nevertheless, when taking into account the EC$_{50}$ (extract concentration causing 50% extract inhibition) values of the aqueous extracts (Table 2), bitter white yam cooked (EC$_{50}$ = 119.1µg/ml) had the highest inhibitory effect on Fe$^{3+}$ induced lipid peroxidation in rat’s brain while bitter white yam raw (EC$_{50}$ = 196.9 µg/ml) had the least.

<table>
<thead>
<tr>
<th></th>
<th>EC$_{50}$ (mg/ml)</th>
<th>DPPH*</th>
<th>EC$_{50}$ µg/ml MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw</td>
<td>Cooked</td>
<td>Raw</td>
</tr>
<tr>
<td>Bitter white yam</td>
<td>5.02</td>
<td>4.68</td>
<td>196.89</td>
</tr>
<tr>
<td>Bitter yellow yam</td>
<td>15.50</td>
<td>16.18</td>
<td>129.12</td>
</tr>
</tbody>
</table>

EC$_{50}$ – Extract concentration that will cause 50% inhibition

CONCLUSION

It is therefore concluded that bitter yams possess antioxidant properties, however white bitter yams are more potent than the yellow bitter yams. Cooking enhanced the antioxidant potentials of the white bitter yams. The observed activities might be as a result of the higher phenolics in these samples.

REFERENCES


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