Effect of Heat Treatment on the Antioxidant Capacity of Aqueous and Ethanol Extracts of *Aframomum angustifolium* Seed

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**ARTICLE INFO**

**ABSTRACT**

Spices are rich in phytochemicals which are responsible for their observed antioxidant property. This potential can be affected by heat treatment when spices are used as food additives. This research studied the effect of heat treatment on the antioxidant capacity of aqueous and ethanol extracts of *Aframomum angustifolium* seed. The antioxidant capacity was measured in vitro by evaluating the metal chelating activity (MCA), ferric reducing ability (FRA), hydroxyl radical (•OH) scavenging activity, nitric oxide (NO) scavenging activity and total antioxidant capacity (TAC) before and after heat treatment. The percentage metal chelating activity of the extracts before heating were comparable (p > 0.05) to that of ascorbic acid while the percentage metal chelating activity of the ethanol extract decreased significantly (p < 0.05) from 65.99% to 47.62% after heat treatment for 30 min. The percentage hydroxyl radical scavenging activity of the aqueous extract was unaffected (p > 0.05) by heat treatment whereas the activity in ethanol extract decreased. The ferric reducing ability of the extracts decreased significantly after heat treatment while the NO scavenging activity increased with heat treatment. The total antioxidant capacity (TAC) of the extracts measured as ascorbic acid equivalents reduced but were not significantly affected by heat treatment. The results of this study suggest that the antioxidant potential of aqueous and ethanol extracts of *A. angustifolium* seeds is not totally lost by heat treatment.

**Introduction**

Thermal treatment has been reported to induce changes in organoleptic, nutritional and textural properties of foods. Cooking increases food safety by destroying food microbial flora, inactivating anti-nutritional factors and enhancing digestibility of foods thereby increasing bioavailability of nutrients. However, cooking and other thermal processes have also been reported to reduce food quality. Thermal processing affects the phytochemicals responsible for the pharmacological properties of plants. There is a general belief that levels of phytochemicals are reduced by thermal treatment. Home cooking methods have been reported to cause significant decreases in the phytochemical contents and antioxidant capacity of faba bean (*Vicia faba* L.). However, an overall increase in antioxidant activity of extracts from *Citrus ushin* peels compared to the unheated samples have also been reported.

*Aframomum angustifolium*, a member of the Zingiberaceae family commonly called Longoza plant is a geophyte which occurs in rain forests, ground-water forests and seasonally dry forests in Africa. Various parts of the plant are used extensively in ethnomedicine. The seeds are used as worm expeller in the Democratic Republic of Congo and the root-stock is given to alleviate postpartum pain in Liberia. The medicinal use of this botanical family as laxatives, anthelmintics, antidiarrhoea and as a tonic for sexual stimulation as well as antiulcer, antimicrobial, antinociceptive,

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Nitric oxide (NO) scavenging assay

The NO scavenging activity was measured using the spectrophotometric method as described by Green et al.\textsuperscript{30} The extract (1 mL, 100 µg/mL) was mixed with 1 mL of sodium nitroprusside solution (5 mM) and incubated at 25°C for 2 h. An aliquot (1 mL) of the reaction mixture was mixed with 1 mL Griess reagent (1% sulfanilamide, 2% phosphoric acid, and 0.1% naphthylethylene diamine dihydrochloride) and the absorbance measured at 546 nm. The nitric oxide scavenging activity of the extracts were also measured as described above after subjecting extracts to heat treatment in a water bath (80°C) for 10 min and 30 min respectively. Ascorbic acid was used as standard. Percentage NO scavenging activity was calculated using the formula:

\[
\% \text{ NO scavenging activity} = \left( \frac{A_0-A}{A_0} \right) \times 100
\]

Where: \(A_0\) is the absorbance of the control (without extract) and \(A\) is absorbance in the presence of extract.

Total antioxidant capacity (TAC)

The TAC was measured using the spectrophotometric method as described by Srivivasan et al.\textsuperscript{31} Aliquots of the test extracts (100 µg/mL) were mixed with 3 mL of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) in test tubes. The tubes were capped with aluminium foil and incubated in a boiling water bath at 95°C for 90 min. The reaction mixture was cooled to room temperature and the absorbance of the solution was measured at 765 nm. The total antioxidant capacity of the plant extract was extrapolated as ascorbic acid equivalent within the range 10 - 100 µg/mL. The effect of heat treatment on the TAC of the extracts were also measured as described above after subjecting extracts to heat treatment in a water bath (80°C) for 10 min and 30 min.

Statistical analysis

All assays were performed in triplicates and results expressed as Mean ± SEM. Experimental data were analysed by one-way analysis of variance (ANOVA) to evaluate significant difference at \(p < 0.05\).

Results and Discussion

Apart from improving the organoleptic property of food, phytochemical components of spices also have antioxidant capacity.\textsuperscript{7} In this study, the effect of heat treatment on the antioxidant capacity of aqueous and ethanol extracts of \textit{A. angustifolium} seeds were investigated \textit{in vitro}. The metal chelating activity of the aqueous extract after heat treatment for 10 min and 30 min was not significantly affected (\(p < 0.05\)) affected while that of the ethanol extract reduced. Metals catalyze oxidative reactions \textit{in vivo}. Reduction/chelation prevents them from participating in redox reactions. Both extracts were observed to be effective chelators of ferrous ion radical compared to ascorbic acid (Table 1). Also, the ferric reducing ability of the aqueous and ethanol extracts were comparable to ascorbic acid before and after heat treatment for 10 min. Heat treatment for 30 min, significantly reduced (\(p < 0.05\)) this ability (Table 2). This suggests that prolonged heat treatment reduces the ferric reducing ability of the extracts. This may negatively impact the ability to inhibit the Fenton reaction. However, a significant increase in the ferric reducing power of citrus peel extracts have been reported after heating for 30 min.\textsuperscript{32} The metal chelating activity of 

Hydroxyl radical scavenging activity (HRSA)

The HRSA was measured using the spectrophotometric method as described by Elizabeth and Rao.\textsuperscript{22,23} The final solution was diluted before reading absorbance. The reaction mixture (3.0 mL) containing 1.0 mL of 1.5 mM FeSO\textsubscript{4}.7H\textsubscript{2}O, 0.7 mL of 6 mM hydrogen peroxide, 0.3 mL of 20 mM sodium salicylate and 1 mL of the extract solution (100 µg/mL) was incubated for 1 h at 37°C. The absorbance of the hydroxylated salicylate complex was measured at 562 nm. The hydroxyl radical scavenging activity of the extracts were also measured as described above after subjecting extracts to heat treatment in a water bath (80°C) for 10 min and 30 min respectively and was calculated using the formula:

\[
\% \text{ Hydroxyl radical scavenging activity} = \left[ 1 - \left( \frac{A_0-A}{A_0} \right) \right] \times 100
\]

Where: \(A_0\) is the absorbance without sodium salicylate. A\textsubscript{1} is the absorbance without sodium salicylate.

It is thus important to ascertain if heat treatment will alter the antioxidative property of \textit{Aframomum angustifolium} seeds.

Materials and Methods

Sample collection and extract preparation

\textit{Aframomum angustifolium} fruits were purchased in Ebbelle market, Igueben Local Government Area of Edo State. The seeds were investigated in the Department of Biological Sciences, Samuel Adegboyega University, Ogwa, Edo State, Nigeria. The seeds obtained were sun dried for five days and thereafter pulverized using a mechanical blender. Two parts (400 g each) were macerated in 800 mL water and ethanol for 72 h with periodic agitation. After extraction, the solutions were filtered with cheese cloth and concentrated using rotary evaporator. The concentrates were dried to powder using silica gel in a desiccator and powder was stored in air-tight bottles at room temperature.\textsuperscript{24}

Assay for Antioxidant Activity

Metal chelating activity (MCA)

The MCA was measured using the spectrophotometric method as described by Dinis et al.\textsuperscript{25,26} The reaction mixture containing 0.5 mL of extract (100 µg/mL), 1.6 mL of deionized water, 0.05 mL of FeCl\textsubscript{2} (2 mM) and 0.1 mL of 1,10-phenanthroline (5 mM) was incubated at 40°C for 10 min and the absorbance measured at 562 nm. Metal chelating activity of the extracts were also measured as described above after subjecting extracts to heat treatment in a water bath (80°C) for 10 min and 30 min. Ascorbic acid was used as standard. The percentage metal chelating activity was calculated using the formula:

\[
\% \text{ Metal chelating activity} = \left[ 1 - \left( \frac{A_1 - A_2}{A_0} \right) \right] \times 100
\]

Where:

- \(A_0\) = absorbance of the control (without extract)
- \(A_1\) = absorbance in the presence of extract
- \(A_2\) = absorbance without FeCl\textsubscript{2}

Ferric reducing ability (FRA)

The FRA was measured using the spectrophotometric principle described by Benzie and Strain.\textsuperscript{27} by mixing reconstituted methanol solutions of extracts (1 mL, 100 µg/mL) with 2.5 mL phosphate buffer (0.2 M, \textit{pH} 6.6) and 2.5 mL potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min. Thereafter, 2.5 mL of 10% trichloroacetic acid was added to the mixtures and then centrifuged at 3000 rpm for 10 min. An aliquot (2.5 mL) of the supernatant was mixed with distilled water (2.5 mL) and 0.1% ferric chloride (0.5 mL). The absorbance of the reaction mixture was measured at 700 nm. The ferric reducing ability of the extracts were also measured as described above after subjecting extracts to heat treatment in a water bath (80°C) for 10 min and 30 min respectively. Ascorbic acid was used as standard. The percentage ferric reducing ability was calculated using the formula:

\[
\% \text{ Ferric reducing ability} = \left( \frac{A_0 - A}{A_0} \right) \times 100
\]

Where:

- \(A_0\) = absorbance of the control (all reagents without extract) and
- \(A\) = absorbance of all reagent with extract

Hydroxyl radical scavenging activity (HRSA)

The HRSA was measured using the spectrophotometric method as described by Elizabeth and Rao.\textsuperscript{22,23} The final solution was diluted before reading absorbance. The reaction mixture (3.0 mL) containing 1.0 mL of 1.5 mM FeSO\textsubscript{4}.7H\textsubscript{2}O, 0.7 mL of 6 mM hydrogen peroxide, 0.3 mL of 20 mM sodium salicylate and 1 mL of the extract solution (100 µg/mL) was incubated for 1 h at 37°C. The absorbance of the hydroxylated salicylate complex was measured at 562 nm. The hydroxyl radical scavenging activity of the extracts were also measured as described above after subjecting extracts to heat treatment in a water bath (80°C) for 10 min and 30 min respectively and was calculated using the formula:

\[
\% \text{ Hydroxyl radical scavenging activity} = \left[ 1 - \left( \frac{A_0-A}{A_0} \right) \right] \times 100
\]

Where: \(A_0\) is absorbance of the control (without extract), \(A\) is the absorbance in the presence of the extract, and \(A_1\) is the absorbance without sodium salicylate.
was significant (p < 0.05) for the aqueous extract. After 30 min, the percentage nitric oxide scavenging activity were not significantly different from that observed for ascorbic acid (Table 4). This suggests that heating may improve the NO scavenging activity of the extract. This possible improvement in NO scavenging activity suggests thermal induction of modifications in the bioactive components with improved NO scavenging ability. High concentrations of NO in vivo is associated with nitrosative stress. This observed improved ability to remove NO in vitro may be replicated in vivo thus preventing its deleterious reactions.

Table 1: Percentage metal chelating activity before and after heat treatment.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Aqueous extract (%)</th>
<th>Ethanol extract (%)</th>
<th>Ascorbic acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>66.82 ± 0.92</td>
<td>65.99 ± 0.06</td>
<td>80.61 ± 1.64</td>
</tr>
<tr>
<td>10</td>
<td>58.37 ± 0.72</td>
<td>63.56 ± 2.66</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>67.44 ± 0.46</td>
<td>47.62 ± 1.55</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 3 assays.

Table 2: Percentage ferric reducing ability before and after heat treatment.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Aqueous extract (%)</th>
<th>Ethanol extract (%)</th>
<th>Ascorbic acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>70.89 ± 0.40</td>
<td>75.48 ± 0.77</td>
<td>96.44 ± 0.64</td>
</tr>
<tr>
<td>10</td>
<td>76.22 ± 0.75</td>
<td>82.07 ± 0.98</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>7.83 ± 0.41</td>
<td>4.48 ± 0.22</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 3 assays.

Table 3: Percentage hydroxyl radical scavenging activity before and after heat treatment.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Aqueous extracts (%)</th>
<th>Ethanol extracts (%)</th>
<th>Ascorbic acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>51.85 ± 0.57</td>
<td>44.64 ± 0.80</td>
<td>76.23 ± 0.11</td>
</tr>
<tr>
<td>10</td>
<td>53.32 ± 0.62</td>
<td>49.56 ± 0.99</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>65.58 ± 0.75</td>
<td>39.25 ± 0.36</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 3 assays.

Table 4: Percentage NO scavenging activity before and after heat treatment.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Aqueous Ext. (%)</th>
<th>Ethanol Ext. (%)</th>
<th>Asc. Acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>33.11 ± 0.53</td>
<td>28.92 ± 0.39</td>
<td>80.08 ± 0.24</td>
</tr>
<tr>
<td>10</td>
<td>90.94 ± 1.13</td>
<td>46.71 ± 0.84</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>86.33 ± 0.23</td>
<td>83.56 ± 0.42</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 3 assays.

The total antioxidant capacity (TAC) of the extracts of A. angustifolium were measured as ascorbic acid equivalents. The overall TAC of the extracts decreased but was not significant (p > 0.05) after heat treatment (Table 5). A decrease in total antioxidant capacity was also observed in a study on the effect of heat treatment on fada beans. The TAC is a unifying concept used to access the antioxidant status of biological samples thus providing an integrated parameter rather than the simple sum of measurable antioxidants. A non-significant reduction of the TAC supports the observation that the antioxidant capacity is not completely lost. The effect of heat treatment on the antioxidative property of plants differ. The observed antioxidant effect is a product of structural modifications of existing compounds or the new compounds formed as a result of thermal processing.

Conclusion
The results of this study indicated that the antioxidant potential of A. angustifolium is not totally lost by heat treatment. These findings are crucial as it provides insight to the use of this spice as food flavourant and the expected benefits of the antioxidative property after thermal processing. Further research into structural changes of bioactive components induced by heat treatment of the extracts as well as in vivo studies to ascertain the effect of metabolic reactions is suggested.

Conflict of Interest
There are no competing financial interests.

Authors’ Declaration
The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements
The authors sincerely appreciate the Department of Chemical Sciences, Samuel Adegboyega University, Ogwa, Edo State, Nigeria for the facilities and laboratory space provided.

References


