# CHANGES IN THE ANTIOXIDANT PROPERTIES OF THE SEED OF *MONODORA MYRISTICA* (*GAERTN.*) DUNAL DURING COOKING

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***Abstract***
*This study aimed at evaluating the influence of cooking temperatures on the antioxidant properties and total phenolic contents of the seed of Monodora myristica (Gaertn.) Dunal. Antioxidant properties of the raw sample and samples cooked for 5 minutes at different temperatures of 40, 70 and 100°C were assessed through 1, 1 diphenyl-2-picryl hydrazyl radical (DPPH•) scavenging properties and ferric ion reducing potentials assays. The estimation of the total phenolic content was carried out using Folin-Ciocalteu reagent, as an index of the antioxidant compounds in the sample. The antioxidant activity significantly decreased ($p<0.05$) after cooking at temperatures 40 and 100°C. However, a sporadic and significant increase was observed in treatment at 70°C. Parallel results were observed through the quantifications of the phenolic content. The results revealed significant changes in the antioxidant properties and the concentration of the phenolics, specifically at a higher temperature. The study suggested that cooking temperature should be reduced to prevent extreme degradation of the essential antioxidant activity and total phenolic content in any requisite aqua-thermal treatment involving the seed of M. myristica.*

**Key words**: *Monodora myristica* (*Gaertn*.) Dunal., cooking, temperatures, antioxidant properties, phenolics, spices.

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## 1. INTRODUCTION

The seed of *Monodora myristica* (*Gaertn*.) Dunal, commonly known as African nutmeg is traditionally used as a spice due to its savoury and aromatic flavour. The seeds are beneficial against the initiation and progression of diseases that are related to oxidative stress (Dorman and Deans, 2004). This corroborates the use of the seed as a dietary spice and also in the treatment and management of different diseases. In general, spices are plant materials commonly used in culinary preparation of local dishes as flavour and aroma inducers. Also, they could enhance food quality as a result of their intrinsic presence of provitamins, vitamins, minerals and variety of phytonutrients with substantial flavour and sensory induced characteristic properties and overall improvement of nutritional qualities. Plant materials have long been known to possess therapeutic potential as they are good sources of dietary antioxidants (Oso *et al*., 2018). Antioxidants are substances that delay or prevent oxidation of substrates that are prone to oxidation. The modes of actions of most antioxidant compounds include inhibitions of initiation and propagation of oxidising chain reactions by free radicals, thus reducing consequent oxidative damage (Halliwell, 2008). The potency of antioxidants from any plant is influenced by factors which include the conditions and processes of extraction and post-harvest handling (Oso *et al*., 2018). Most spices are subjected to thermal treatment before being consumed. Many studies had reported thermal processes could cause impulsive changes in the antioxidant potentials of plant materials. For example, the antioxidant capacities and total phenolic contents of broccoli, white cabbage, cauliflower, decreased significantly by thermal treatment (Shams El-Din *et al*., 2013; Kalkan and Yücecan, 2013; Amin and Lee, 2005; Ali, 2015). Conversely, some studies reported that cooking could enhance the antioxidant potentials of plant materials such as black pepper (*Piper guineense* Schum and Thonn), Negro pepper (*Xylopia aethiopica* [Dun.] A. Rich.), eggplant (*Solanum melongena* L.), spider plant (*Cleome*

*gynandra* L.) after thermal treatment (Miglio *et al.*, 2008; Nwozo *et al.*, 2015; Zambrano-Moreno *et al.*, 2015). Cooking procedures, time and temperature have been emphasised through various studies as factors that could contribute to the unpredictable effect of cooking on antioxidant capacities of any plant material (Yamaguchi *et al.*, 2001; Oso and Oladiji, 2019). This study was aimed at investigating the influence of different cooking temperatures on the antioxidant properties of the seed of *M. myristica*.

## 2. MATERIALS AND METHODS

### Chemicals

All chemicals were of analytical grade. 1, 1-diphenyl-2-picrylhydrazyl (E-Labscience, China), Ethanol, sodium dihydrogen phosphate and disodium hydrogen phosphate (Guangzhou *JHD Chemical* Reagent Co., China) sodium carbonate, Gallic acid, potassium ferricyanide, acetic acid, Folin Ciocalteu's phenol reagent (Loba Chemie, India).

### Preparation of plants samples

The seeds of *M. myristica* were obtained from local suppliers in Ogunmakin, Ogun State, Nigeria. The plant materials were authenticated at the Department of Biological Sciences, McPherson University, Seriki Sotayo, Nigeria. The edible parts were removed from the outer shell and pulverised using ceramic mortar and pestle. The pulverised samples were divided into four different portions, each comprising 100 g of the sample added to 250 ml of distilled water in a 500 ml conical flask. A portion was retained as raw and the other three portions were randomly assigned to different groups that were cooked separately at temperatures of 40°C, 70°C and 100°C for 5 minutes using a water bath (SUB Aqua plus Grant REX-C700, England). The sample preparation and cooking procedures were repeated three times. The raw and the cooked samples were then homogenized and filtered using Whatman No.1 filter paper. The filtrates were stored in the refrigerator (-4 °C) for the subsequent analyses.

### Estimation of total phenolic content

The total phenolic content of each extract was estimated using Folin-Ciocalteu reagent (FCR)

### Determination of 1, 1 diphenyl-2-picryl hydrazyl radical (DPPH$^{\bullet}$) scavenging ability

The determination of DPPH$^{\bullet}$ scavenging ability of the sample was carried out as described by Shirwaikar *et al.* (2006) based on the reduction of purple DPPH$^{\bullet}$ to a yellow coloured diphenyl picrylhydrazine. The reaction mixture contained 0.5 ml of each extract and 1.0 ml solution of 0.1 mM DPPH$^{\bullet}$ freshly prepared in absolute methanol. The mixture was allowed to stand in the dark for 20 minutes and the absorbance was subsequently read at 517 nm using a spectrophotometer (General Scientific, GS-UVII). A corresponding mixture containing methanol in place of an extract was used as the blank. The percentage of inhibition was calculated according to the following equation:

$$\% \text{ inhibition} = [(\text{Absorbance of blank} - \text{Absorbance of sample}) / \text{Absorbance of blank}] \times 100$$

### Determination of ferric ion reducing potential

The ferric reducing potential of each extract was carried out as described by Oyaizu (1986). A reaction mixture containing 1.0 ml of each sample, 1.0 ml of freshly prepared 0.2 M sodium phosphate buffer (pH 6.6) and 1.0 ml of 1% potassium ferricyanide was incubated at 50 °C for 15 minutes. Thereafter, 50 µl of 5% trichloroacetic acid solution was added to the mixture and centrifuged at 650 ×*g* using an 800-D centrifugal machine for 5 minutes. Exactly, 1.0 ml of the supernatant was cautiously transferred into a test tube containing 0.1 ml of 0.1% ferric chloride solution. The absorbance was read at 700 nm. Ascorbic acid was used as the standard with the concentration ranging between 10 and 50 µg/ml.

as described by Singleton *et al.* (1999) based on the reduction of the FCR by the phenols in the extract. A reaction mixture containing 0.1 ml of each extract and 0.5 ml of the FCR was

allowed to stand at a temperature of 30°C for 40 minutes. Subsequently, 1.4 ml of 7.5% sodium carbonate solution was added to the mixture and made up to 5.0 ml using distilled water. The absorbance was read at 765 nm after 60 minutes. Gallic acid was used as the standard with the concentration ranging between 10 and 50 µg/ml.

**Statistical analysis**

The data obtained were subjected to statistical analyses using a one-way analysis of variance. The results were expressed in mean ± standard deviation of three determinations and differences between the means were examined by Duncan's New Multiple post-hoc tests using the IBM SPSS Statistics 20 software.

## 3. RESULTS AND DISCUSSION

The scavenging of radical (DPPH$^{\bullet}$) capacity decreased significantly ($p<0.05$) in the cooked samples when compared to the raw (Figure 1). However, the DPPH$^{\bullet}$ scavenging potential of the sample cooked at 70 °C was significantly ($p<0.05$) higher compared to the sample cooked at 40 °C. Sample cooked at 100°C had the lowest antioxidant potential. Likewise, changes in the ferric ion reducing potential of the sample were observed when cooked at 100 °C. The reducing potential of the filtrate obtained from sample cooked at 100 °C significantly reduced ($p<0.05$) compared to others (Figure 2). The effect of cooking on total phenolics was shown in Figure 3. The total phenolics of the cooked sample decreased significantly at $p<0.05$. The total phenolics decreased in the order of raw > cooked at 70 °C > cooked at 100 °C > cooked at 40 °C.

The vacillating effect of temperature on the radical scavenging property and ferric ion reducing potential of the sample could be related to the natural release of antioxidant compounds at slightly elevated temperature followed by thermal decomposition of the released antioxidant compounds at a higher temperature. Thermal degradation of antioxidant compounds and inactivation of antioxidant enzymes are usually associated with decreased antioxidant potential plant products (Hager *et al*., 2010). These observations were not in agreement with some previous studies that emphasised the enhancement of antioxidant potential of some plant materials through cooking (Dewanto *et al*., 2002; Adefegha and Oboh, 2011; Nwozo *et al.*, 2015). The deviation could be related to the distinctiveness of each plant in terms of phytochemical compositions.

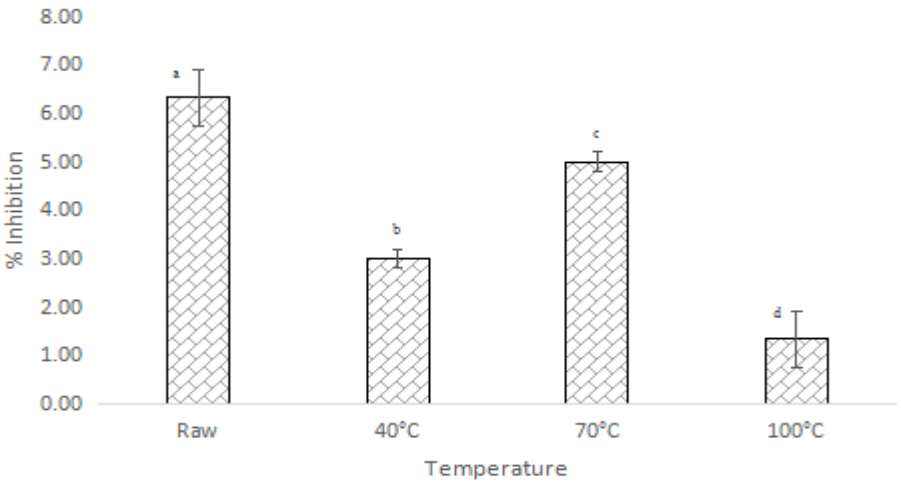


**Figure 1: Percentage DPPH$^{\bullet}$ scavenging ability of raw and cooked samples of the seed of *M. myristica*. Different alphabets indicate a significant difference at the level $p<0.05$**

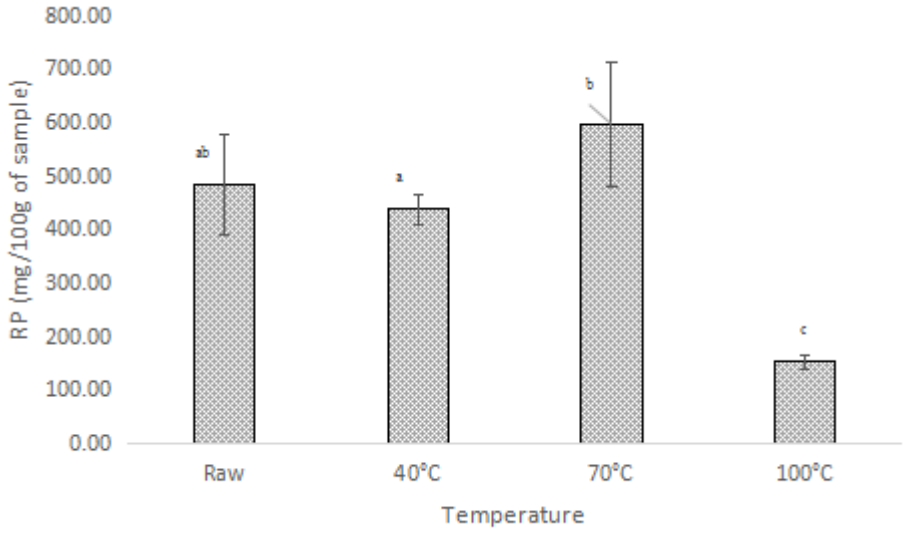


**Figure 2: Reducing potentials (RP) of raw and cooked samples of the seed of *M. myristica*. Different alphabets indicate a significant difference at the level p<0.05**

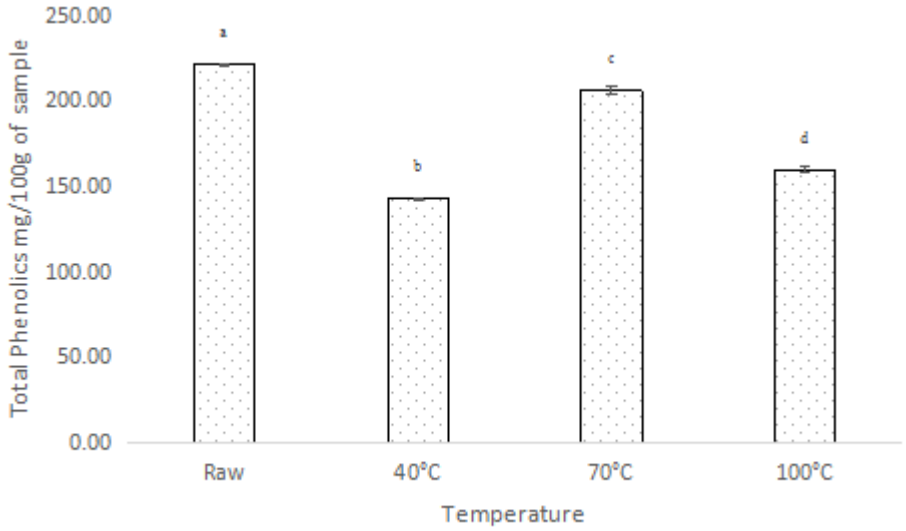


**Figure 3: Total phenolics of raw and cooked samples of the seed of *M. myristica*. Different alphabets indicate a significant difference at the level p<0.05**

The changes that occurred in estimations of the total phenolic content of the sample were similar to the apparent antioxidant capacities. The chemistry and activity of phenolic compounds could be influenced by the quantity, the stability and the interaction of the phenolic compounds with other bioactive compounds. Other factors that could influence the effect of cooking on the quantity of the phenolic content include the structures and the natures of the plant matrix. Phenolics can exist in plants as either free phenolics which are solvent extractable as they exist loosely within the matrix of the plant material or bound phenolics which are covalently bound within the plant matrix and can only be extracted following hydrolysis. Additionally, there could be entrapped phenolics which are firmly but not covalently bound to plant matrix and could be included in the non-extractable phenolics. Thus, the cooking procedure could have a determining factor in dictating the forms and amounts of phenolics obtained in an extract of plant material (Kajdzanoska *et al.,* 2011). These could lead to the release of different

bioactive phenolics with different antioxidant mechanisms.

Previous studies showed that processing methods involving the use of high temperatures or freezing could cause the release of bound or entrapped bioactive compounds from the matrix of plant cell walls and membranes (Harbaum *et al*., 2007; Oso and Oladiji, 2019). These observations could be associated with rupturing of cell walls, hence increasing the bioavailability of the bound or entrapped phytochemicals. Conversely, the present study revealed that the amount of the total phenolics in the sample decreased at high temperature. This could be attributed to the transformation of the thermal-extractable free phenolics in the sample to secondary compounds with lesser apparent antioxidant activity.

Moreover, the reduction in the total antioxidant activity and total phenolics could be partly due to the heat lability of compounds present in *M. myristica*. The biological properties of *M. myristica* had been associated through various reports to the presence of volatile compounds which include α-pinene, α-phellandrene, β-pinene, elemicin, isoelemicin, myrcene, myristicin and sabinene and they contribute to the antioxidant properties of the spice (Takikawa *et al.*, 2002; Dorman and Deans, 2004; Narasimhan and Dhake, 2006). Cooking could have decomposed the phytochemicals which could contribute to the antioxidant capacity of the raw extract as these volatile compounds could be fairly unstable and easily degraded because of changes of temperature. Consequently, the volatility of these compounds could, however, contribute to the reduction in the chemical antioxidant capacities of the sample at different temperatures.

## 4. CONCLUSIONS

The results of the different assays showed that the antioxidant and scavenging potential of the seed of *M. myristica* decreased with increased temperature of cooking. The study further revealed that the antioxidant components were very unstable when exposed to a cooking temperature of nearly 100°C. The potential negative influence of cooking on these compounds could affect the biological potentials of this spice. Thermal processing methods involving the aqueous extract of the seed of *M. myristica* should be limited to mild temperatures to avoid losses of the overall antioxidant activity and/or total phenolics as observed through this study.

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