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The influence of varying cooking temperatures on the antioxidant properties of the aqueous extract of *Piper guineense* (Schumach & Thonn) seeds

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ABSTRACT

The study assessed the influence of varying cooking temperatures on the antioxidant properties of the aqueous extract of *Piper guineense* seeds. Different portions of the aqueous extract of *P. guineense* seeds were cooked at different temperatures which include 50 ºC, 70 ºC, and 90 ºC for 10 minutes, while the remaining portion was allowed to stand for 10 minutes at room temperature of 29 ºC and all were assessed for the evaluations of reducing power, radical scavenging capacities, and total phenolic contents. The results showed that there was a significant increase in the ferric reducing power at 90 ºC, however, there was no significant difference between the raw and the cooked *P. guineense* seeds at 50 ºC and 70 ºC. In addition, the radical scavenging potential of the extract was highest at 70 ºC, however, none of the cooked *P. guineense* seeds at the selected temperatures exhibited lower DPPH scavenging property. A similar trend was observed for the phenolic content of the extract with a significant reduction at 90 ºC. The study suggests that cooking at 70 ºC could enhance the antioxidant potentials of *P. guineense* seeds.

Introduction

Free radicals are substances carrying unpaired electrons synthesized in small amounts, possessing the ability to attack another with no unpaired electron, causing oxidative damage to cellular macromolecules such as proteins, DNA, and lipids, leading to increased risk of degenerative diseases (Rezaeizadeh et al., 2011). However, free radicals can be beneficial to biological systems and the obvious examples include their role in immune defence, antibacterial action, vascular tone, signal transduction, etc. (Gutowski, 2013). Medicinal plants are said to be a rich source of antioxidants, which are an effective and persuasive alternative with few and transient side effects in curing and managing ailments (Pandey et al., 2011; Sofowora et al., 2013; Ortega-Ramirez et al., 2014; Guan and He, 2015). Medicinal plant extracts (as well as herbal/spices) contain different non-nutrient compounds with a variety of biological behaviour of valuable therapeutic indices. These ameliorating and protective effects of plants are associated with the activities, concentration, and potentials of phytochemicals (Poongothai et al., 2011; Etim et al., 2013). Herbs, spices, and other plant parts have been used in many different ways. Since the past few decades, there has been a tremendous increase of interest in using dietary antioxidants, especially from plant sources, to reduce the risk of degenerative diseases such as cardiovascular disease, cancer, and immune dysfunction (Chun et al., 2005; Maritess et al., 2005). This emerging interest had been associated with high antioxidant activity of some of the spices and their beneficial effects on human health (Charles, 2013; Choi et al., 2014; Alexander et al., 2017; McCormick, 2020). Antioxidants can be defined as any molecule capable of stabilizing or terminating the effects of free radicals in the cells. Antioxidants could exhibit a beneficial role in maintaining human health by inhibiting or delaying free radical damage in the

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body (Rahman, 2007). A large group of phytoconstituents such as phenolic compounds, sulphur-containing compounds, tannins, alkaloids, phenolic diterpenes, and vitamins from spices has been proved to possess antioxidant properties (Peter, 2001; Peter, 2004; Parthasarathy et al., 2008; Charles, 2013; Yesiloglu et al., 2013; Choi and Cha, 2014; Srinivasan, 2014; McCormick, 2020). *Piper guineense* (Schumach & Thonn), belongs to a family of Piperaceae. It is found in the tropical region of Central and Western Africa and it is a climbing perennial plant commonly known as West African black pepper. *Piper guineense*, with indigenous name *Iyere* in Yoruba and Uziza in Ibo, similar to cubeb pepper in terms of flavour but fresher and less bitter, is widely consumed as a spice in food and its fruits contain essential oil and pungent piperine constituting 5 to 8 % of the weight of black pepper. It is therefore used in the beverage and pharmaceutical industries as flavouring and as a preservative agent (Opara, 2014; Oyemitan et al., 2014; De LaTorres et al., 2017). The spiciness of the pepper is due to the presence of resins, predominantly chavicine and the yellow alkaloid (Hassan et al., 2010). The reported medicinal beneficial effects of *P. guineense* include keeping the body warm and prevention, cold morning sickness, allergy treatment, and prevention (Ekanem et al., 2010; Tankan and Ito, 2013; Etim et al., 2013; Opara, 2014).

While fruits are mostly consumed in their raw form, vegetables are usually consumed after cooking, performed in different ways, either according to personal tastes or culinary traditions. Food processing is a set of methods and techniques applied towards the transformation of raw ingredients into food for consumption by humans and animals. This is done not only to improve flavour, digestibility, and palatability of foods but also to increase food safety, either by the destruction of microorganisms and/or inactivation of antinutritional factors, such as the inactivation of polyphenol oxidase (Palermo et al., 2014).

However, biological, chemical, and physical properties of vegetables are modified during thermal food processing, causing changes in flavour, texture, or colour, and most indispensably, its effects on the concentration of non-nutrient compounds and bioavailability (such as the release of free polyphenols from fibre-bound polyphenols) in vegetables (Palermo et al., 2014). Interestingly, these modifications depend upon the differences in the employed methods of food processing and morphological and nutritional characteristics of vegetable species (Nicoli et al., 1999; Lee and Kader, 2000; Miglio et al., 2008; Sengul et al., 2014).

Therefore, the present study was carried out to evaluate the effect of varying cooking temperature on total phenolic content, antioxidant potentials, and DPPH-radical scavenging activity of *P. guineense* seed aqueous extract, to study the possibility of utilizing the plant in the treatment and management of disorders.

Materials and methods

Materials

Absolute methanol and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from JHD in China and the other reagents were of analytical grade. Fresh seeds of *P. guineense* were purchased from Ogunmakin market, Obafemi/Owode Local Government, Ogun State, Nigeria and authenticated at the Department of Biological Sciences, McPherson University at room temperature of 30±1 °C. Filter paper (Whatman grade 1) and an electrical thermostatic water bath were used, and all spectrophotometric analyses were performed on the GS UV11 Spectrophotometer.

Methods

Sample preparation

Exactly 5.0 g of the dried sample was ground to a fine powder and extracted with 50 mL of distilled water for 120 minutes at room temperature of 29 °C. The dried sample was ground to a fine powder (15.0 g) and extracted with 150 mL of distilled water at 50 °C, 70 °C, and 90 °C. The samples were filtered on Whatman TM No 1. The filtrate was used for the assessment of phenolic contents, ferric reducing power, and radical scavenging capacities.

Ferric Reducing Power Assay

The reducing property of each cooked extract and the raw extracts were determined spectrometrically as described by Oyaizu (1986). The extract (2.5 mL) was mixed with 2.5 ml of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min and then 2.5 mL of 10 % trichloroacetic acid (TCA) was added. This mixture was subjected to centrifugation at 650 rpm for 10 min, 5 ml of the supernatant was mixed with an equal volume of water, and 1 mL of 0.1% $FeCl_3$. The absorbance was read at 700 nm. The ferric reducing antioxidant potential was subsequently calculated using ascorbic acid as the standard and the result was expressed

in ascorbic acid equivalent mg per 100 g of the fresh sample.

Determination of Percentage of Radical Scavenging Capacity

The radical-scavenging potential of each cooked extract and raw extracts were assessed as described by Oso and Ogidi (2019), using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical as the source of the free radical. Exactly 2.0 mL of each extract was mixed with 1.0mL of 100 µM DPPH solution prepared in methanol and the mixture was left in the dark for 20 minutes. The absorbance was measured at 517 nm and the percentage of radical scavenging capacity was calculated relative to that of the control without the extract according to the equation:

$$Percentage\ Inhibition = \frac{A0 - A1}{A0} \times 100$$

A0 = the absorbance of the control; *A1* = the absorbance of the sample.

Determination of total phenolic content

The quantity of TPC was estimated using the Folin–Ciocalteau reagent (Singleton et al., 1999). Precisely 1.0 mL of the Folin–Ciocalteau reagent (1:10 v/v) was added to 0.5 mL of the sample and left to stand at room temperature for 20 minutes, thereafter, 5 mL of 10% (w/v) Na_2CO_3 was added. The absorbance was read at 725 nm after 10 minutes. The quantity of the total phenolics was calculated as garlic acid equivalent (GAE) and expressed in mg per 100 g of dried weight (DW).

Statistical Analysis

The obtained results were expressed as means ± standard error of the mean of three determinations and analysed using one-way variance analysis (ANOVA) for mean differences between treatments, followed by the Duncan multiple range test for post-hoc correlation at $p<0.05$, using SPSS version 16.0, SPSS Inc, Chicago, IL.

Results

The influence of temperature on the antioxidant properties of *P. guineense* seeds is presented in Table 1. The results showed that the reducing power of fresh and cooked *P. guineense* seeds at different temperatures (50 °C, 70 °C, and 90 °C). Cooking at these respective temperatures (50 °C and 70 °C) caused no significant differences ($p<0.05$) in the reducing power activity of *P. guineense* seeds. However, at 90 °C, a significant increase ($p<0.05$) in the reducing power potential (595.07±91.92 mg/100 g DW) of *P. guineense* seeds was observed.
In addition, no significant difference ($p<0.05$) was observed between DPPH scavenging activity of raw *P. guineense* seeds and cooked *P. guineense* seeds at 50 °C. However, a significant increase ($p<0.05$) in DPPH scavenging potential of *P. guineense* seeds was observed at higher temperatures and the increase is temperature-dependent. The total phenolic content of raw *P. guineense* seeds was significantly different ($p<0.05$) from the cooked *P. guineense* seeds at all temperatures (Fig. 1). The cooking of *P. guineense* seeds reduced the total phenolic content at all temperatures except at 70 °C, where a significant increase in total phenolic content was seen (Fig. 1).

Table 1. Ferric ion reducing activity (mg/100g DW) and DPPH radical scavenging activity (%) of *P. guineense* seeds and at different cooking temperatures

Extracts	FRAP mg/100g DW)	DPPH (%)
RPGAE	299.45±26.60^{a}	9.83±0.64^{a}
PGAE 50	432.66±19.23^{ab}	11.16±0.67^{ab}
PGAE 70	477.37±68.74^{ab}	20.41±0.17^{b}
PGAE 90	595.07±91.92^{b}	12.15±0.77^{c}

Values are expressed as the mean of 3 replicates ± standard error of the mean. Values with different superscripts within a column are significantly different ($p<0.05$). PGAE= *P. guineense* aqueous extract, RPGAE= Raw *P. guineense* aqueous extract, PGAE 50= *P. guineense* aqueous extract at 50 °C, PGAE 70= *P. guineense* aqueous extract at 70 °C, PGAE 90= *P. guineense* aqueous extract at 90 °C and DPPH= 2, 2-Diphenyl-1-picrylhydrazyl.

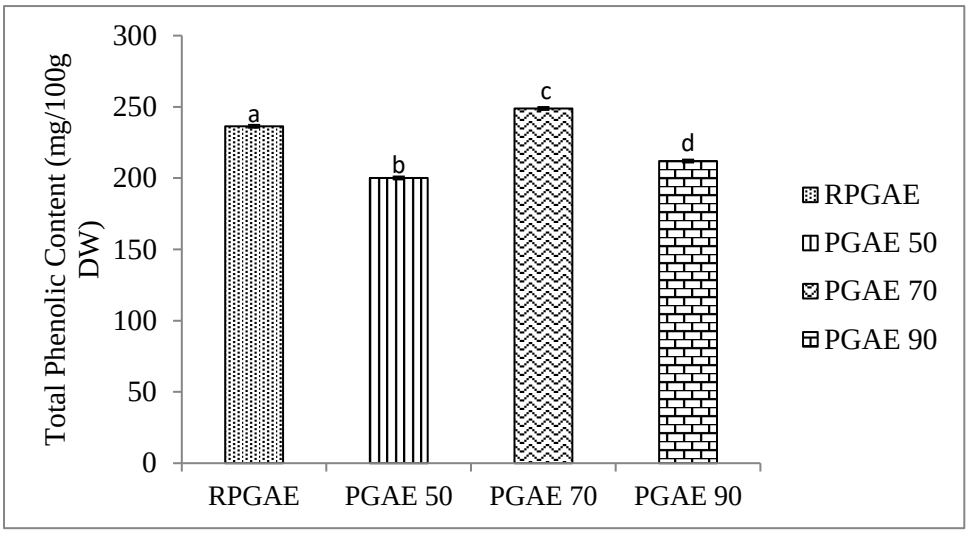

Fig. 1. Total phenolic content (mg/100g DW) of *P. guineense* seeds and at different cooking temperatures. PGAE= *P. guineense* aqueous extract, RPGAE= Raw *P. guineense* aqueous extract, PGAE 50= *P. guineense* aqueous extract at 50 °C, PGAE 70= *P. guineense* aqueous extract at 70 °C, PGAE 90= *P. guineense* aqueous extract at 90 °C

Values are expressed as the mean of 3 replicates ± standard error of the mean. Values with different superscripts on the bar are significantly different ($p < 0.05$). PGAE= *P. guineense* aqueous extract, RPGAE= Raw *P. guineense* aqueous extract, PGAE 50= *P. guineense* aqueous extract at 50 °C, PGAE 70= *P. guineense* aqueous extract at 70 °C, PGAE 90= *P. guineense* aqueous extract at 90 °C and TPC= Total phenolic content.

Discussion

Antioxidants, either exogenous or endogenous, are vital substances that exhibit a protective effect on the body from damage caused by free radical-induced oxidative stress (Ghasemzadeh and Ghasemzadeh, 2011). Research has shown that exogenous antioxidants can be obtained from synthetic products such as butylated hydroxyanisole, butylated hydroxytoluene, and tertiary butylhydroquinone, which have been used extensively in foods or plant foods which are a rich source of non-nutrient compounds to prevent oxidation. However, synthetic antioxidants used as additives in foods had been discouraged because of their toxicities and carcinogenicities (Zhang et al., 2016). Interestingly, natural antioxidants such as flavonoids, tannins, and phenolics found in various plant parts/products such as fruits, leaves, seeds, and oils (Faller and Fialho, 2009) are presumed safe with equal or better antioxidant potential than synthetic antioxidant values (Thaiphong et al.,2006; Doss and Pugalenthi, 2012; Kumar and Pandey, 2013). Conversely, most plant foods are consumed after food processing such as boiling, frying, roasting, to mention but a few, for better digestion and metabolism in the human digestive system. Food processing mainly involves boiling with different energy transfer media such as air, soil, water, and electromagnetic waves (Nayak et al., 2015). Convincing evidence has shown that cooking of plant foods could cause a mixed effect on non-nutrient compounds responsible for therapeutic effects, such as the antioxidant potential of plant foods. Therefore, the present study has investigated the effect of different cooking temperatures on *P. guineense* seed aqueous extract to evaluate the concentration and bioavailability of phytoconstituents in *P. guineense* seeds responsible for its antioxidant properties.

The result of the study indicates that cooking contributed to an increase in the mean, reducing the potential of *P. guineense* seeds compared to the raw sample, even at a temperature that is as high as 90 °C. However, this report of significant increases at a high temperature (90 °C), which is not in agreement with Mayeaux et al. (2006), who reported that an increase in time with the cooking temperature of 90 °C could be responsible for the decrease in activity and stability of some phytochemicals. Also, the increase in ferric reducing activity could be due to a high concentration of iron content, a prooxidant in *P. guineense* seeds (Imo et al., 2018) that is temperature dependant.

Phenolic compounds, phenolic acids, and polyphenols are the main class of secondary metabolites in plants that are linked to carbohydrates (Harbone et al., 1999). Several reports have shown a pungent and positive correlation between total phenolic content and antioxidant potentials (Reddy et al., 2010; Sarawong et al., 2014). To substantiate the antioxidant potential

of *P. guineense* seeds, the total phenolic content was evaluated and the result shows that TPC decreases from 236.±1.08 to 200.12±1.08 mg/100g DW at 50 °C. This could imply that the condition could favour the activity of phenoloxidase, which could promote the oxidation of phenol and in turn reduce the phenolic content. This result agrees with the report of Perla et al. (2012), who found that thermal processing reduced phenolic content in food. At 70 °C, there was a 5.29% increase in phenolic content compared to raw *P. guineense* seeds. The increase could be a result of an increase in temperature inducing the release of phenolic compounds from the bound carbohydrates due to the rupture of the cell wall (Harbaum et al., 2007) or due to their high solubility in water (Ahmed and Ali, 2013). Also, this could be due to the inhibition of phenoloxidase activity (Chakraborty et al., 2015) or tissue dehydration (Schweiggert et al., 2006). In opposition, the antioxidant potential of *P. guineense* seeds reduced significantly at 90 °C and could be due to the destruction in piperidine, an important oil responsible for pungent antioxidant properties of *P. guineense* seeds via the increased bioavailability of other non-nutrient compounds (Shoba et al., 1998).
Besides, the radical scavenging capacity of *P. guineense* seeds was evaluated and the result showed better radical scavenging potential at all boiling temperatures compared to the raw extracts. Interestingly, the radical scavenging potential of *P. guineense* seeds observed at 50 °C, 70 °C, and 90 °C are respectively 13.53%, 107.63%, and 23.60% higher than the raw extract. This result commensurates with the phenolic content of *P. guineense* seeds boiled at 70 °C where more of it is released. This result corresponds with the already established claim that the total phenolic content corresponds to the antioxidant properties (Reddy et al., 2010; Sarawong et al., 2014).

Conclusion

The present study concludes that phenolic content and antioxidant potentials of *P. guineense* seeds were cooking temperature-dependent. Boiling at the temperature of 70°C increased the phenolic content, the ferric reducing potential, and radical scavenging capacity in the seeds. However, the total phenolic content decreased at a higher temperature of 90°C thus suggesting 70°C as the optimum cooking temperature for the consumption of *P. guineense* seeds as a spice for individual or commercial purposes.

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