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RESEARCH PAPER

COOKING MODULATES THE ANTIOXIDANT ACTIVITIES OF RHIZOMES OF *ZINGIBER OFFICINALE* ROSCO AND *CURCUMA LONGA* L.

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ABSTRACT

This research was aimed at investigating the influence of cooking on the anti-oxidative potentials of the rhizomes of Zingiber officinale Rosco and Curcuma longa L. The investigations were performed on both raw and cooked aqueous infusions of the rhizomes. The antioxidant potentials were assessed through the estimations of oxidant scavenging capacities, lipid peroxidation inhibition potential and reducing capacities. Further investigations were carried out on the phytochemical analyses of the extracts through the assessments of phenolic and flavonoid contents. The results revealed that the radical scavenging capacities of the infusions of the rhizomes when cooked reduced statistically ($p<0.05$). Similar changes were observed in the nitric oxide scavenging activities and inhibition of lipid peroxidations of both extracts. On the contrary, the phenolics in the infusions were enhanced. This study demonstrated that the impact of cooking on the antioxidant properties of these rhizomes could be determined by the nature of the phytocomponents and probable thermally-induced changes occurring during cooking.

Keywords: Cooking, ginger, turmeric, antioxidant, anti-inflammation

INTRODUCTION

Spices have been established to have medicinal properties due to their health benefits which are associated with their antioxidant, anti-inflammatory, antimicrobial, hypolipidaemic, anti-mutagenic and anti-carcinogenic activities (Shan *et al*. 2005; Oso and Oladiji 2019). They are excellent sources of dietary antioxidants. They contribute to the defence mechanisms of living organisms against the pathologies related to the attacks of free radicals. Intake of plant-derived antioxidants has been hypothesised to be involved in the prevention of diseases that are associated with oxidative stress (Huang *et al*. 2005; Mao *et al*. 2018; Oso *et al*. 2019). Different types of plant-based foods had shown extensively unpredictable antioxidant effectiveness probably due to differences in the handling techniques and the molecular structures of their bioactive compounds. Cooking is one of the major food processes that have been associated with the vacillating effectiveness of antioxidant components in dietary plants. This has led to conflicting reports among scientists on the impact of cooking on the antioxidant components of food products. The conflicting reports could be assumed to be initiated by the unpredictable thermally-induced transformations in the components and structures of the chemicals in the samples (Nwozo *et al*. 2015; Oso and Oladiji 2019).

The rhizomes of *Zingiber officinale* Rosco (ginger) and *Curcuma longa* L. (turmeric) are commonly used as spices due to their flavours enhancement and their acclaimed biological activities (Srivastava *et al*. 2006; Schwetner and Rios 2007; Salmon *et al*. 2012; Dugasani *et al* 2010). These spices have shown to be beneficial against the initiation and progression of various diseases such as rheumatoid arthritis (Ramadan *et al*. 2011). Typically, they are incorporated into the culinary preparations which could involve thermal treatments. This study is aimed at investigating the influence of cooking on the antioxidant capacities of *Z. officinale* and *C.longa*.

MATERIALS AND METHODS

Chemicals

All chemicals were of analytical grade. Gallic acid, quercetin, 1, 1- diphenyl-2-picrylhydrazyl (E-Labscience, Wuhan, China), sodium nitroprusside, sodium carbonate, potassium ferricyanide, acetic acid, Folin Ciocalteu's phenol reagent (Loba Chemie, Mumbai, India).

Preparation of plant samples

Fresh rhizomes of *Zingiber officinale* Rosco and *Curcuma longa* L. 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. Fifty grams of each sample were transferred separately into a 500 mL flat-bottom flask containing 200 mL of distilled water. The mixtures were homogenized and filtered. The filtrates, in each flask, were put on a heating mantle at temperatures of 60°C and 100°C and cooked for 10 min and 20 min each. These temperatures were chosen because of their applications in culinary preparations involving spices (Oso and Olaoye, 2020). The filtrates were stored at -18°C for subsequent analyses. Moreover, the filtrates of the raw samples were also subjected to the proposed analytic procedures.

Ascorbic acid equivalent antioxidant capacity

The ascorbic acid equivalent antioxidant capacities (AEAC) of the samples were carried out using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) discolouration assay as described by Oso and Ogidi (2019). Precisely 0.5 mL of each extract was transferred into a test tube containing 0.5 mL of 0.05 M phosphate buffer

(pH 7.0). Thereafter, 2.0 mL of 0.1 mM DPPH• recently prepared in methanol was added into the tubes. The absorbance was read at 517 nm after 20 min of incubation in the dark at a room temperature of 29˚C. The antioxidant capacity of each extract was calculated from the standard curve obtained from ascorbic acid discolouration activity. The results were expressed in µg/g of dry weight.

Nitric oxide scavenging activity

Nitric oxide radical scavenging activity was determined through the method reported by Garrat (2006). Precisely 2.0 mL of 1% sodium nitroprusside prepared in 0.1 M phosphate buffer (pH 7.4) was added into a test tube containing 0.1 mL of the reconstituted extract and the mixture was incubated for 60 min at room temperature of 30°C. Afterwards, 0.5 mL of the incubated mixture was pipetted into another test tube containing 1.0 mL sulphanilic acid reagent prepared by adding 33% sulphanilic acid to 20% glacial acetic acid and incubated at room temperature for 5 min. Lastly, 1.0 mL of 0.1 % naphthyl ethylenediamine dihydrochloride was added and incubated at room temperature for 30 min before taking the absorbance at 540 nm with a spectrophotometer. The percentage inhibition was calculated using;

$$\%\text{inhibition} = \frac{Ao - A1}{Ao} \times 100,$$

where Ao = Absorbance of the control and A1= Absorbance of extract. The inhibition concentration at 50% (IC50) was calculated and expressed in mg/mL.

Inhibition of lipid peroxidation

The determination was carried out as described by Ruberto *et al*. (2000), using egg yolk homogenate (2.0% v/v) as the source of the lipid as described by Oso *et al*. (2019). Accurately, 0.1 mL of freshly prepared egg yolk solution was added to a test tube containing 0.5 mL of 0.1 M phosphate buffer (pH 9.0) and 0.5 mL of the extract. Afterwards, 0.05 mL of ferrous sulphate was added and the mixture was allowed to incubate at 29°C for 5 min. Subsequently, 0.5mL of acetic acid-thiobarbituric acid reagent prepared in dimethyl sulphoxide was added to the mixture and kept in a boiling water bath for 60 min. The solution was allowed to cool and centrifuged at 650 ×g for 5 min and the absorbance of the supernatant was measured at 532 nm. The percentage inhibition was calculated using;

$$\%\text{inhibition} = \frac{Ao - A1}{Ao} \times 100,$$

where Ao = Absorbance of the control and A1= Absorbance of extract. The inhibition concentration at 50% (IC_{50}) was calculated and expressed in mg/mL.

Ferric reduction antioxidant potential

The ferric reduction antioxidant potential of each extract was estimated based on the reduction of potassium ferricyanide to potassium ferrocyanide as described by Oyaizu (1986). Precisely 0.25 mL of each filtrate was added to 2.5 mL of 0.1 M phosphate buffer (pH 6.6). Subsequently, 0.5 mL of 0.5% (w/v) potassium ferricyanide was added. The reaction mixture was allowed to incubate at room temperature (29°C) for 30 min. Later, 0.1 mL of 5% trichloroacetic acid was added and the mixture was centrifuged at 600×g for 5 min. An equal volume of the supernatant (1.0 mL), deionized water and 0.1% ferric chloride solution were mixed in a separate test tube and incubated for 30 min. The absorbance was subsequently measured at 700 nm. The reducing activity of each sample was calculated from the standard curve obtained from varying concentrations of ascorbic acid and expressed in µg/g of dry weight.

Total phenolic contents

The total phenolics of each extract were estimated using the Folin-Ciocalteu reagent (FCR) 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 29°C for 40 min. 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. Then the absorbance at 765 nm was measured against a blank which contained all the reagents without the samples. The phenolic content of each sample was calculated from the standard curve obtained from varying concentrations of Gallic acid and expressed in µg/g of dry weight.

Statistical analysis

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

RESULTS AND DISCUSSION

Assessment of *in vitro* antioxidant properties

Assessment of the *in vitro* antioxidant properties revealed that cooking at 60°C and 100°C for 10 and 20 min significantly decreased the ascorbic acid antioxidant equivalents of both infusions of *Z. officinale* and *C. longa* except when *Z. officinale* was cooked at 60°C for 20 min though there was no significant difference ($p<0.05$) (Table 1). These results were based on the proton donating potentials of the infusions and discolouration of DPPH•. This report was contrary to the previous reports that suggested that cooking could enhance the antioxidant properties of plant materials (Faller and Fialho, 2009; Nwozo *et al*. 2015). However, many of these earlier studies were carried out on samples that were exposed to thermal treatment before extraction. The observation in this study could be due to the thermal degradation of antioxidant compounds and the inactivation of antioxidant enzymes in the aqueous infusion (Hager *et al*., 2010; Diaconeasa *et al*., 2017). The slight elevation in the AEAC of the infusions of both rhizomes at 60°C when cooked for 20 min could be associated with thermal modifications of pytocompounds with antioxidant potential or inactivation of the polyphenol oxidase (Lee *et al*., 2009). Examples of phytocompounds that could be influenced by thermal processes include chlorogenic acid, kaempferol-rutinose, and rutin (Blessington *et al*. 2010). This observation corresponds with the report of Lachman *et al*. (2012) on the elevation of anthocyanin content in *Solanum tuberosum* L. when subjected to thermal treatment. Besides, the nitric oxide scavenging properties assessed through the Griess Illosvoy reaction after cooking showed a considerable reduction in the aqueous infusions of the plant materials. The nitric oxide scavenging potential of the infusion of *Z. officinale* significantly decreased when cooked at 60°C for 20 min and at 100°C for 10 and 20 min (Table 1) as shown by the comparatively higher half-maximal inhibitory concentration (IC_{50}).

Table 1. Ascorbic acid antioxidant equivalent (AEAC) and nitric oxide scavenging potentials of raw and cooked aqueous infusions of *Z. officinale* and *C. longa*.

Cooking Temperature (°C)	Cooking Time (mins)	AEAC (µg/g)		NO scavenging potential (IC_{50} mg/ml)	
		Z. Officinale	*C. longa*	*Z. officinale*	*C. longa*
Raw		406.67±19.14^{a}	314.00±1.00^{a}	295.33±43.18^{a}	236.67±7.04^{a}
60	10	359.33±23.35^{b}	187.00±20.78^{b}	309.00±43.86^{a}	213.33±38.73^{a}
	20	416.00±11.53^{a}	98.33±18.67^{c}	304.00±9.16^{a}	345.33±148.67^{b}
100	10	377.00±6.03^{b}	301.33±14.15^{d}	339.33±43.59^{b}	397.33±20.50^{b}
	20	290.67±21.38^{c}	194.54±18.70^{b}	624.32±21.38^{c}	453.48±26.74^{c}

The values represent the mean ± standard deviation of three determinations. Different alphabets within a column show the values are statistically different at p<0.05.

Similarly, the lipid peroxidation inhibition potential of *Z. officinale* decreased significantly when cooked at 60°C for 10 and 20 min and at 100°C when cooked for 10 min. Thus, cooking could lead to a detrimental decrease in lipid peroxidation inhibition potential of the infusion of *Z. officinale*. Conversely, it was observed that the lipid peroxidation inhibitory potential of the extracts of *Z. officinale* increased at 100°C when cooked for 20 min. Moreover, there was a general increase in the lipid peroxidation inhibitory potential of the extracts of *C. longa*. The stability of ferric ion reducing potential which is the direct measure of total antioxidant potential of the infusions based on electron transfer appears to be favourably enhanced at higher temperatures in both infusions. Cooking significantly increased the ferric reducing potential of *Z. officinale*; nevertheless, there was no significant effect on the influence of cooking at 60°C and 100°C for 10 and 20 min of the aqueous infusion of *C. longa* (Table 2).

Table 2. Lipid peroxidation inhibition and ferric ion reducing potentials of raw and cooked aqueous infusions of *Z. officinale* and *C. longa.*

Cooking Temperature (°C)	Cooking Time (mins)	Inhibition of lipid peroxidation (IC_{50} mg/ml)		Ferric ion reducing potential (µg/g)	
		Z. officinale	*C. longa*	*Z. officinale*	*C. longa*
Raw		414.21±14.73^{a}	299.66±5.50^{a}	88.33±1.52^{a}	32.33±2.08^{a}
60	10	467.00±5.196^{b}	257.67±3.05^{a}	102.00±1.73^{b}	28.00±0.57^{a}
	20	452.36±8.55^{b}	241.00±3.00^{b}	95.33±2.08^{c}	31.00±5.19^{a}
100	10	455.00±19.05^{b}	256.67±14.22^{c}	103.00±1.00^{b}	27.33±0.58^{a}
	20	342.33±13.01^{c}	251.00±7.81^{c}	104.67±2.08^{b}	70.33±1.52^{b}

The values represent the mean ± standard deviation of three determinations. Different alphabets within a column show the values are statistically different at p<0.05.

Previous studies had reported partial degradation of antioxidant compounds in spices when exposed to longer heat (Hager *et al*., 2010; Oso and Olaoye, 2020); it is however evident from this study that, cooking could retain and increase antioxidant activities of these spices. This could corroborate the earlier observations that cooking could not be exclusively detrimental to all categories of plants with regard to the antioxidants potentials (Ng *et al*. 2011; Hossain *et al*. 2017; Tan *et al*. 2018). The observed increase in the antioxidant properties could be attributed to the breakdown of complex polyphenols into simple polyphenols (Pellergrini *et al*., 2009). In addition, an increase in the dissolution power of the solutes together with a probable reduction in oxidative deterioration of the extract during cooking could be responsible for the enchanced reducing properties through the inhibition of lipid peroxidation and ferric ion reducing potential. Similarly, the observed dissimilarities in the responses of these infusions could be related to differences in the phytochemical components of the rhizomes (Schwetner and Rios, 2007; Qin *et al*. 2007; Paramasivam *et al*. 2009; Salmon *et al*. 2012). It is important to note that this observation with reference to inhibition of lipid peroxidation and ferric ion reducing potential is not consistent with the reported observation through AEAC and nitric oxide scavenging potentials where a decrease in the antioxidant capacities was documented; thus, the type of experimental model used could also account for variances in the outcomes of this study. For example, the outcomes of the method used for the determination of the reduction ability ability of Fe^{3+} to Fe^{2+} had been shown to depend on specific conditions which should be optimised for each contributor of the antioxidant properties (Wojtunik-Kulesza, 2020); the indeterminate heat-induced changes in the natures of these antioxidant compounds during cooking could induce false reduction potentials.

Amount of phenolic contents

The effects of thermal degradation on the bioactive compounds of the rhizomes were evaluated through estimations of the total phenolic contents of the infusions. Previous studies on the evaluation of phenolic profile of *Z. officinale* revealed the main phytoconstituents of this rhizomes which could be responsible for the acclaimed beneficial properties including gingerols, gingerdiols, paradols, shogaols, and zingerone (Mishra and Palanivelu, 2008; Wilson *et al*., 2013). Curcumin and turmerone were shown to be major pharmacologically active phytochemicals in *C. longa* (Sharma *et al*., 2005; Cheng *et al*., 2012). The results are presented in Table 3. Cooking at 60°C for 20 min significantly decreased the total phenolic contents of the infusion of *Z. officinale*; conversely, an increase in the temperature to 100°C at 10 and 20 min significantly enhanced the phenolic contents. The effects of domestic cooking on polyphenolic contents of the plant materials have been found to depend on various factors which include the cooking method, the nature of the food sample, the chemical nature of the polyphenol and the methods of analysis (Faller and Fialho, 2009; Nwozo *et al*., 2015; Oso and Oladiji, 2019; Wu *et al*., 2019). Besides, the classical Folin-Ciocalteu assay for the quantification of total phenolics could be influenced by various reducing substances presenting in the infusion other than phenolic acids and flavonoids (Sanchez-Rangel *et al*., 2013). Examples of reducing substances possessing antioxidant abilities are Maillard reaction products which could be formed when foods containing reducing sugars and amino groups are cooked or processed at high temperatures. This could account for the unusual higher increase level of the quantified phenols in the aqueous extract of *Z. officinale* at 100°C.

Table 3. Amount of phenolic contents in raw and cooked aqueous infusions of *Z. officinale* and *C .longa*

Cooking Temperature (°C)	Cooking Time (mins)	Phenolic contents (µg/g)	
		Z. officinale	*C. longa*
Raw		62.00±3.00[a]	64.32±2.04[a]
60	10	63.00±1.73[a]	55.33±0.57[b]
	20	53.00±1.00[b]	54.33±0.57[b]
100	10	72.00±0.57[c]	63.00±0.50[a]
	20	74.33±0.57[d]	65.33±0.57[a]

The values represent the mean ± standard deviation of three determinations. Different alphabets within a column show the values are statistically different at $p<0.05$

CONCLUSION

The present study on the influence of cooking on the antioxidant properties of the rhizomes of *Z. officinale* and *C. longa* revealed that durations of cooking and temperature could have variable effects on the antioxidant properties of the rhizomes which could be associated with the type of the experimental procedure, phytoconstituents of the rhizhomes and probable thermally-induced changes of the natures of the contributors of antioxidant capacity. Further studies are recommended to identify specific changes induced during cooking of these plant materials.

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DISCLOSURE STATEMENT

There is no conflict of interest in this work.

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