Free Radicals and Antioxidants. 2018; 8(2):89-95
A Multifaceted Journal in the field of Free Radicals and Antioxidants
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Original Article

Phytochemical Composition and Antioxidant Potential of Oven Heated and Microwave Treated Ginger (*Zingiber officinale* Roscoe)

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ABSTRACT

Objective: The study was designed to investigate the effect of heating in the simple oven and microwave treatment on phytochemical composition and antioxidant potential of ginger (*Zingiber officinale* R.), a popular food ingredient of medicinal importance. **Methods:** Dried ginger was ground to fine powder and subjected to heating in the simple oven and microwave oven in an increasing order of heating time (5, 10, 15, 20 and 25 min). The heat-treated samples were extracted in methanol and analyzed for their phytochemical composition and antioxidant potential. **Results:** A time-dependent significant ($p<0.05$) negative effect of both treatment methods was observed on total phenolic acids (TPA), total tannins content (TTC), total flavonoids content (TFC), total antioxidant activity (TAOA), 2, 2-diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH RSC) and hydroxyl radical scavenging capacity (HRSC). Both treatments methods showed the significant positive effect on β-carotene bleaching capacity (BCBC), reducing power (RP) and iron chelating activity (ICA). TPA and TAOA of oven heated, TTC and RP of microwave-treated and TFC, BCBC, and HRSC of ginger varied exponentially while TTC and RP of the oven heated, TPA and TAOA of microwave-treated and ICA and DPPH RSC showed linear variation in response to treatment time. **Conclusion:** Both heating methods have a significant time-dependent effect on the phytochemical composition and antioxidant activity of ginger. This study provides useful information to the manufacturers and consumers regarding the potential benefits of ginger and effect of various heat treatments methods on its medicinal value.

Key words: Antioxidant activity, β-carotene bleaching assay, Free radical scavenging capacity, Ginger (*Zingiber officinale*), Microwave treatment.

Key message: Ginger is a popular ingredient used in the preparation of numerous foods and has a valuable part in pharmaceutical formulations. The heating treatments during the cooking and pharmaceutical processing were hypothesized to affect the nutritional and medicinal value of ginger. The hypothesis was proved to be true and the study has shown that the heating methods have a significant time-dependent effect on the phytochemical composition and antioxidant activity of ginger. The study would be helpful to the manufacturers and consumers in selection of suitable heating methods for ginger-based food recipes and pharmaceutical processing to avoid the loss of its biological potential.

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History
- Submission Date: 28-11-2017;
- Review completed: 10-02-2018;
- Accepted Date: 28-05-2018.

DOI : 10.5530/fra.2018.2.14

Article Available online
http://www.antiox.org/v8/i2

INTRODUCTION

Ginger, botanically known as *Zingiber officinale*, is a spice used as an ingredient in the preparation of numerous food and pharmaceutical formulations. It also possesses numerous medicinal properties due to the presence of bioactive phytochemical compounds. It has been used in Chinese, Ayurvedic and Tibb-Unani medicines since ancient times for treatment of many diseases including arthritis, sprains, muscular aches, pains, sore throats, constipation, indigestion, vomiting, hypertension, dementia, fever, infectious diseases and helminthiasis.[1] Ginger has been reported to possess anti-obesity, anti-microbial, anti-fungal, anti-thrombotic, anti-inflammatory, anti-hepatotoxic, and anti-cancer activities. It has been also found to be useful in curing heart diseases, cancers, arthritis and Alzheimer's disease.[1-5] Ginger is a good source of antioxidant compounds which protect the biomolecules from oxidative damage such as protein denaturation, lipid peroxidation and DNA lesions caused by endogenous free radicals produced during various metabolic processes.[6] Ginger has been reported to decrease age-related oxidative stress markers and to suppress oxidative stress, lipid peroxidation, NO production, and superoxide production.[3,5,7] Ginger, due to its

Cite this article: Nawaz H, Shad MA, Aziz AR, Muntaha ST, Muzaffar S. Phytochemical Composition and Antioxidant Potential of Oven Heated and Microwave Treated Ginger (*Zingiber officinale* Roscoe). Free Radicals and Antioxidants. 2018;8(2):89-5.

antioxidant potential, has also been reported to normalize the superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione-S-transferase activities in rats.[5]

The processing techniques significantly influence the nutritional and pharmaceutical value, phytochemical composition and antioxidant properties which further influence the medicinal value of vegetables and spices.[8,9] The cooking method significantly influences the content and activity of bioactive compounds present in vegetables and spices. Boiling, steaming in simple and microwave ovens are the commonly used method considered safe for the preparation of vegetable-based good quality food and pharmaceutical products. Steaming the foods in a simple and microwave oven in the covered and thermostable container helps in preventing the loss of micronutrients and phytochemicals from plant material during processing.[10,11] The microwave heating has been reported to affect the extraction yield of bioactive phytochemical compounds from plant materials and their antioxidant activity.[12,13,14] However, some studies have shown that the heating treatments result in a temperature-dependent decrease in phytochemical composition and antioxidant activity of ginger.[15-17]

The present study was designed to study the comparative time-dependent effect of heating in the simple oven, a conventional heating method, and steam-free heating in microwave oven on the phytochemical composition antioxidant activity of ginger. The data would be a significant contribution to current research on the thermal and microwave stability of phytochemicals in ginger.

MATERIALS AND METHODS

Ginger rhizome was purchased from the local market, washed in distilled water, cut into pieces with a sharp knife and dried under shade in the flow of fresh air until constant weight and minimum moisture content (2.5-3%). The dried sample was ground to a fine powder using an electric grinder (National Juicer Blender & Grinder JPN 176, Japan) at low speed (1000 rpm) to avoid temperature fluctuation during grinding. The ground sample proceeded for oven heating and microwave treatment.

Oven heating and Microwave Treatment

The ground sample was subjected to oven heating and microwave treatments each at five treatment times (5, 10, 15, 20 and 25 min). The treatment times were selected after an initial experiment in the range prior to combustion of the sample. The oven heating was done in a simple oven at a constant temperature (150°C) while microwave treatment was done in a microwave oven (Microwave-Orient-OMW-720-ADL). The operating conditions for microwave treatment were selected as radiation intensity: at medium intensity (Power level P50: 400W, Equivalent to 150-160°C), sample mass per load: 5g, treatment duration: as selected by experimental design. The treatment was discontinued after each 5 min for 30 secs and the sample were mixed thoroughly to avoid the burning.

Preparation of Extracts

After heat treatment, the samples were soaked in 80% methanol (sample to the solvent ratio 1:20) and kept at room temperature (25±3°C) in dark for 24 h. The extract was obtained by filtration and evaporation of the solvent. The solvent-free extract was stored in glass bottles covered with aluminum foil in at sterile and thermally controlled standard laboratory conditions. The dried extract (0.1g) was dissolved in methanol (100 ml) and proceeded for phytochemical and antioxidant analysis. The chances of photo-oxidation of phytochemical compounds were minimized by protecting the samples from direct sunlight exposure throughout the study period.

Phytochemical Analysis

Total phenolic acids

Total phenolic acids (TPA) in ginger were estimated by the method of by Slinkard and Singleton.[18] The total phenolic acids were calculated as gallic acid equivalent (g/100 g dry weight using the linear regression equation [TPA (g/100 g dw.) = Absorbance at 720 nm/0.485 + 0.089] from the standard curve of gallic acid (*R2 = 0.974*).

TPA (g/100g dw) = Absorbance at 720 nm/0.485 + 0.089

Total tannins content

Total tannins content (TTC) was estimated by following the vanillin assay.[19] Tannin contents were calculated as gram catechin equivalent (g/100 g dry weight) using following regression equation obtained from a standard curve of catechin (*R 2= 0.994*):

TTC (g/100g dw) = Absorbance at 500 nm/0.361 + 0.005

Total flavonoids contents

Total flavonoid content (TFC) was estimated according to the previously reported method[20] with slight modification. Methanolic extract (1 mL) was diluted to 4 mL with methanol followed by the addition of 2% sodium nitrite solution (0.3 mL). After 6 min 10%_Aluminum nitrate solution (0.3 mL) and 4% sodium hydroxide solution (4 mL) was added and allowed to stand at 25±2°C for 12 min and absorbance was measured at 510 nm against blank. The contents of total flavonoids were calculated as g/100 g dry weight using regression equation obtained from a standard curve of catechin (R^2=*0.995*).

TFC (g/100g dw) = Absorbance at 510 nm/0.146 + 0.008

Antioxidant Analysis

Total antioxidant activity by phosphomolybdenum assay

Gallic acid equivalent total antioxidant activity (TAOA) was evaluated by Phosphomolybdenum assay.[21] This method is based on the reduction of Mo (VI) to Mo (V) by antioxidants and the formation of a green phosphate/Mo (V) complex λ_{max} at 695nm. Total antioxidant activity was calculated as mg equivalent/100 g of gallic acid using regression equation [TAOA (g Eqv./100 g gallic acid) = Absorbance at 695nm/1.25 − 0.13] obtained from the standard curve of gallic acid (R^2 =*0.995*).

TAOA (gallic acid Eqv.g/100g dw) = Absorbance at 695 nm/1.25 − 0.13)

Reducing power

The reducing power (RP) of samples was determined by the method of Oyaizu (1986). A higher absorbance indicates a higher reducing power.

Iron chelation ability

Iron chelating ability (ICA) of extracts was estimated by a modified method described.[22] The inhibition of Fe (II) complex formation was calculated as:

$$ICA\ (\%) = [A_0 - A_1/A_0] \times 100$$

Where A_0 = absorbance control and A1 = Absorbance of the mixture containing the extract.

β-carotene bleaching capacity

The antioxidant activity of samples was also determined in β-carotene linoleate system using reported method [23] and β-carotene bleaching capacity (BCBC) of the extracts was calculated as:

$$BCBC\ (\%) = [Abs_{120}/Abs_0] \times 100$$

Where Abs_{120min} is absorbance of the mixture after 120 min of assay and Abs_0 is absorbance of initial β-carotene.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging capacity

DPPH is a free radical which is stable and contains nitrogen. It has been widely used for determination of the free radical scavenging ability of antioxidants. DPPH radical absorbance is at 517nm. The antioxidants react with DPPH radical, converted into corresponding hydrazine and the fall in extinction is correlated with the potential of antioxidant to scavenge free radicals. The hydrogen donating power of the extracts of ginger was measured spectrophotometrically in terms of the change in color of DPPH solution from purple to yellow due to the inhibition of the DPPH. DPPH radical scavenging capacity (DPPH RSC) was estimated by the method described earlier.[24] The radical scavenging capacity was calculated as:

$$\text{DPPH RSC (\%)} = [Abs_{control} - Abs_{sample}/Abs_{control}]$$

Hydroxyl radicals scavenging ability

Hydroxyl radical scavenging ability (HRSC) of extracts was estimated according to the procedure described by Smirnoff and Cumbes [25] with some modification. Methanolic extract (1 mL) was mixed with 9mM/L ferrous sulfate solution (1 mL) and 9mM/L salicylic acid (1 mL). Hydroxyl radical formation was initiated by the addition of 8.8 mM/mL H_2O_2 (1 mL). The solution was kept in dark for 30 min at 37°C. Absorbance was observed at 510 nm against a blank (without salicylic acid). The control solution was prepared without a sample. The capacity of extracts to scavenge hydroxyl radical was calculated as:

$$\text{HRSC (\%)} = 1 - (Abs_{sample} - Abs_{blank})/Abs_{control} \times 100$$

Statistical Analysis

The results were expressed as the mean ± standard deviation of three parallel replicates. Significant variation among the results obtained at different treatment times was analyzed by one-way analysis of variance (ANOVA). The data were analyzed by regression analysis to study the effect of heat treatment on phytochemical composition and antioxidant properties.

RESULTS

The results of phytochemical composition, antioxidant activity and free radical scavenging capacity of methanolic extracts of untreated ginger are presented in Table 1. TPA, TTC, and TFC were found to be 0.68, 0.28 and 3.9 g/100 g dw respectively. Antioxidant activity of the ginger extract was determined in terms of gallic acid equivalent TAOA by phosphomolybdenum assay, BCBC, RP, and ICA which were found to be

Table 1: Phytochemical composition, antioxidant activity and free radical scavenging capacity of methanolic extracts of untreated ginger.

Phytochemical composition (g/100g extract)	
Total phenolic acids	0.68±0.034
Total tannins content	0.28±0.012
Total flavonoid content	3.93±0.47
Antioxidant activity	
Total antioxidant activity (g/100g dw)	1.54±0.26
β-carotene bleaching capacity (%)	11.76±1.14
Reducing power (Abs. at 700 nm)	0.02±0.003
Iron chelating ability (%)	8.03±0.95
Free radical scavenging capacity (%)	
DPPH radical scavenging capacity	85.88±3.46
Hydroxyl radical scavenging capacity	37.36±2.73

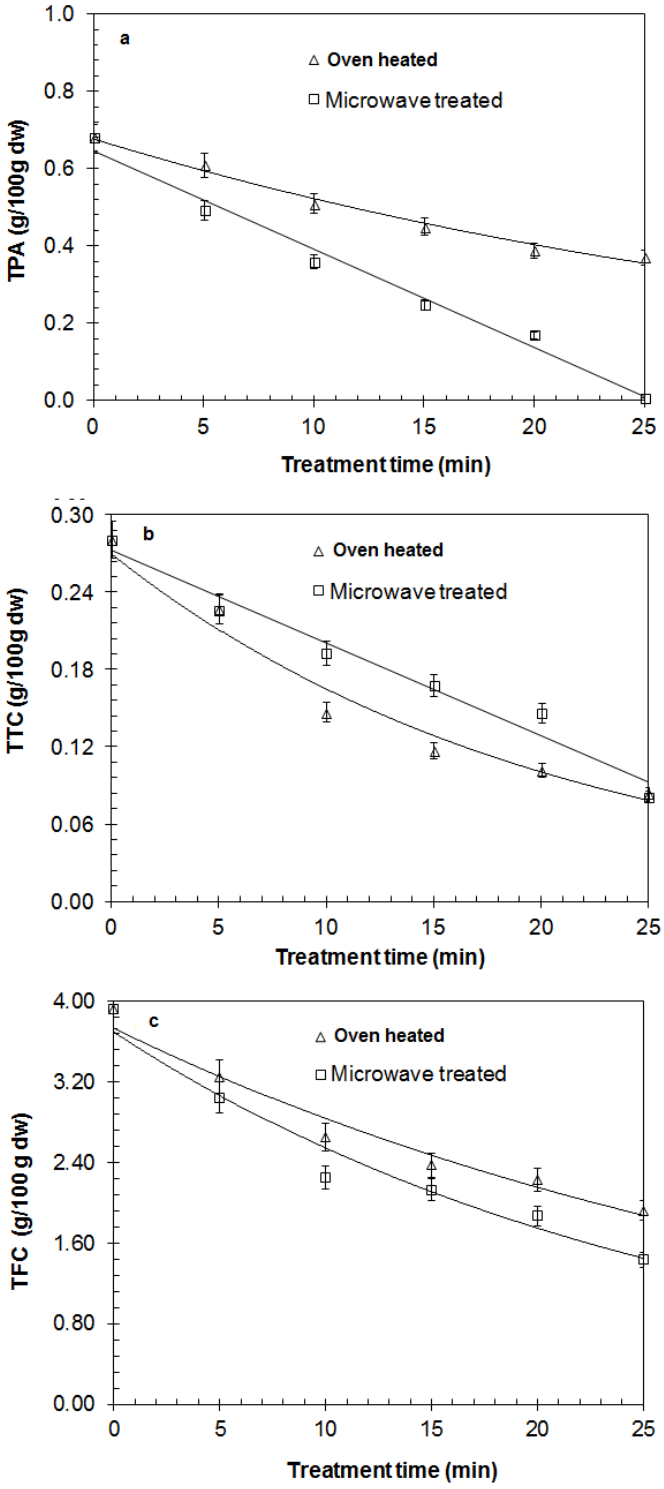

Figure 1: Time-dependent response of a) total phenolic acids, b) total tannins content and c) total flavonoids content of methanolic extracts of oven heated and microwave treated ginger.
*Error bars show the standard deviation of three parallel replicates.

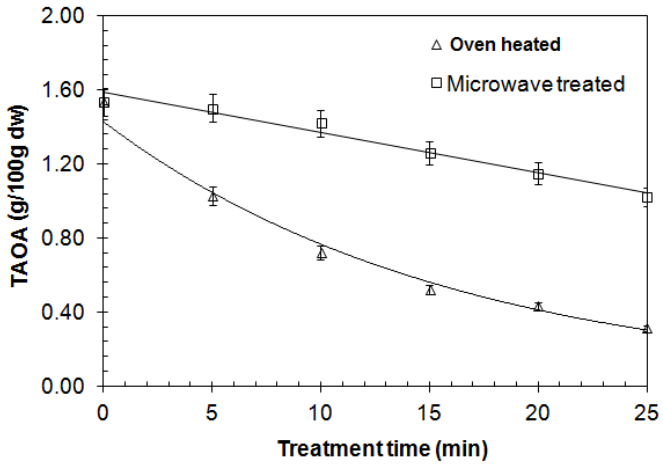

Figure 2: Time-dependent response of Gallic acid equivalent total antioxidant activity (TAOA) of methanolic extracts of oven heated and microwave treated ginger.
*Error bars show the standard deviation of three parallel replicates.

1.54 g/100 g dw, 11.76%, 0.02 (in terms of absorbance at 700 nm) and 8.03% respectively. The ability of ginger extract to inhibit the free radicals, determined in terms of DPPH RSC and HRSC, found to be 85.88 and 37.36% respectively.

Ginger was heated in the simple oven and microwave oven to study the effect of oven heating and microwave treatment on its phytochemical composition, antioxidant activity, and free radical scavenging capacity. The regression analysis of the data showed statistically significant variation ($p<0.05$) in all the studied parameters of ginger subjected to either treatment method. A significant decrease in phytochemical content, total antioxidant activity and free radical scavenging capacity and an increase in metal reducing, metal chelating and β carotene bleaching abilities was observed in response to an increase in treatment time (Table 2). TPA, TTC, and TFC of an oven heated and microwave treated ginger was found to be decreased from 0.68 to 0.007, 0.28 to 0.082 and 3.93 to 1.44 g/100 g dw respectively in response to an increase in treatment time (Figure 1a-c). The oven heating of ginger showed an exponential decrease in TPA and TFC and a linear decrease in TTC (R^2 = 0.969–0.996) while microwave treatment resulted in a linear decrease in TPA and an exponential decrease in TTC and TFC (*R2 = 0.971–0.989*).

Gallic acid equivalent TAOA in an oven heated and microwave treated ginger was found to be decreased from 1.54 to 0.31 and 1.54 to 1.02% respectively (Figure 2). BCBC of both the oven heated and microwave treated ginger was increased from 12-31 and 12-25% respectively (Figure 3a). RP and ICA of oven heated ginger were increased from 0.02-0.13 and 8.03 to 16% while those of microwave-treated ginger was increased from 0.02 to 0.09 and 8.03 to 29% respectively (Figure 3b, c). Effect of oven heating was found to be exponential on TAOA and BCBC and linear on RP and ICA (R^2 = 0.965–0.996). However, the microwave treatment resulted in the linear effect on TAOA and ICA and an exponential increase in BCBC and RP (R^2 = *0.962–0.997*). Table 2.

DPPH RSC of the oven heated, and microwave treated ginger was found to be decreased from 85.88 to 40.96 and 85.88 to 20.99% respectively while HRSC was decreased from 37.36 to 4.49 and 37.36 to 14.40% respectively with an increase in treatment time (Figure 4a, b). Both treatments showed the linear negative effect on DPPH RSC and exponential negative effect on HRSC (R^2 = *0.974–0.985*).

DISCUSSION

Ginger is used as a spice in various foods in the world, particularly in continental and Chines recipes, to enhance the taste and quality of the food products. It is also used as an active ingredient of food due to its important biological activities. It is widely used in pharmaceutical formulations due to the presence of antioxidant phytochemicals of medicinal importance. The nutritional and medicinal quality of plant material entirely depends on the processing conditions and methods. Various processing methods such as boiling, frying, oven heating and microwave treatment have been adapted for the preparation of various food and pharmaceutical products. Microwave heating has been found

Table 2: Regression equations, regression coefficients and p-values of obtained from the regression. Analysis of experimental data.

	Regression equation		Regression coefficient		p-value	
	Roasted	MT[a]	Roasted	MT	Roasted	MT
Phytochemical composition (g/100g dw)						
TPA	$T_{PA} = 0.676\ e^{-0.02t}$	$T_{PA} = 0.645 - 0.025t$	0.982	0.985	0.01	0.00
TTC	$T_{TC} = 0.272 - 0.007t$	$T_{TC} = 0.269e^{-0.04t}$	0.969	0.971	0.00	0.00
TFC	$T_{FC} = 3.737\ e^{-0.02t}$	$T_{FC} = 3.813e^{-0.03t}$	0.996	0.991	0.00	0.00
Antioxidant activity						
TAOA (g/100g dw)	$T_{AOA} = 1.429e^{-0.06t}$	$T_{AOA} = 1.584 - 0.021t$	0.989	0.970	0.03	0.00
BCBC (%)	$BC_{BC} = 11.39e^{0.039t}$	$BC_{BC} = 10.87e^{0.031t}$	0.996	0.968	0.00	0.00
RP (Abs. at 720nm)	$R_P = 0.025 + 0.004t$	$R_P = 0.015e^{0.066t}$	0.966	0.965	0.01	0.02
ICA Activity (%)	$I_{CA} = 8.498 + 0.303t$	$I_{CA} = 8.696 + 0.882t$	0.972	0.969	0.00	0.01
Free radical scavenging capacity (%)						
DPPH RSC	$DPPH_{RSC} = 87.61 - 1.663t$	$DPPH_{RSC} = 90.87 - 2.614t$	0.985	0.976	0.00	0.00
HRSC	$H_{RSC} = 37.22e^{-0.09t}$	$H_{RSC} = 34.72e^{-0.03t}$	0.980	0.974	0.01	0.00

[a]MT: Microwave treated

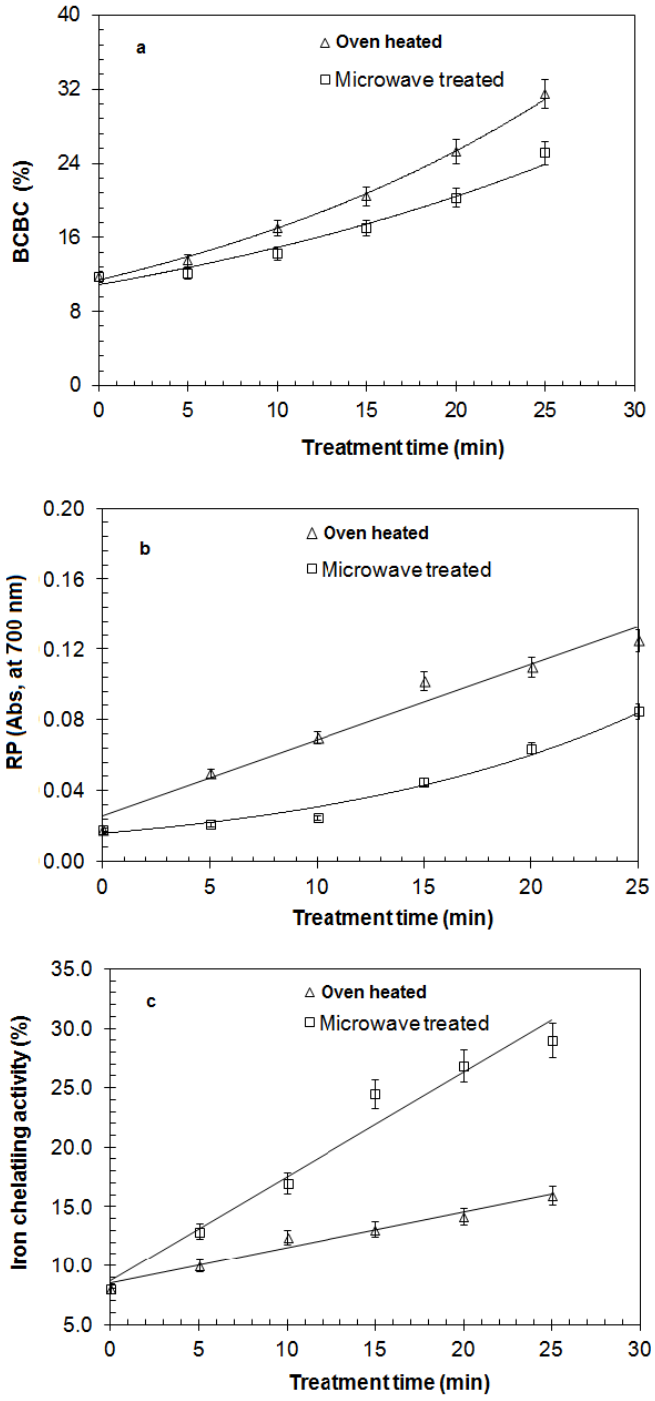

Figure 3: Time-dependent response of a) β-carotene bleaching capacity (BCBC), b) metal reducing power (RP) and c) iron chelating activity (ICA) of methanolic extracts of an oven heated and microwave treated ginger.
*Error bars show the standard deviation of three parallel replicates.

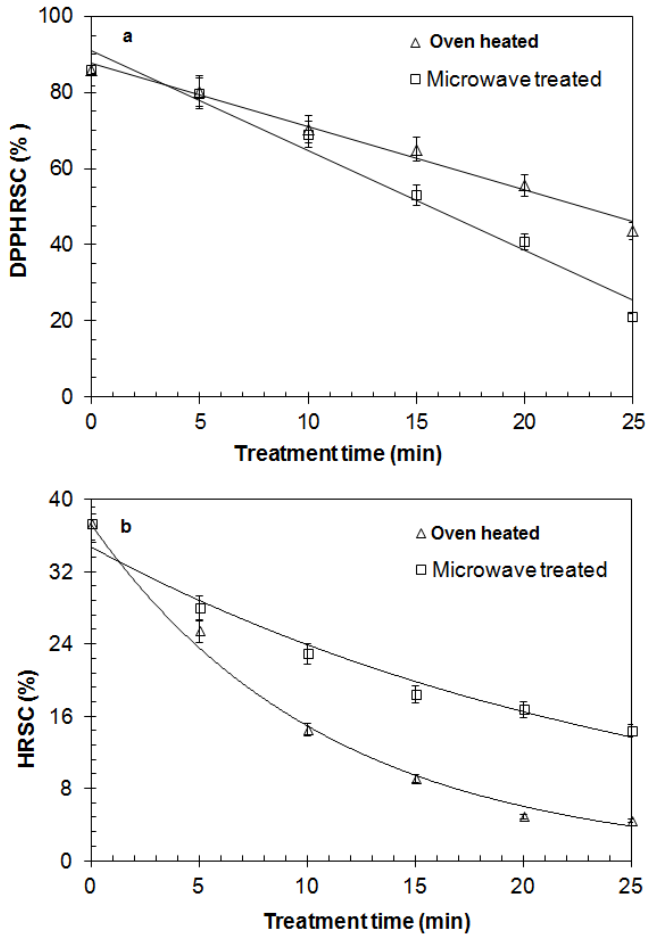

Figure 4: Time-dependent response of a) DPPH radical scavenging capacity (DPPH RSC) and b) hydroxyl radical scavenging capacity (HRSC) of methanolic extracts of oven heated and microwave treated ginger.
*Error bars show the standard deviation of three parallel replicates.

to be effective in improving the nutritional quality of starch and protein-based food products and phytochemical and antioxidant based pharmaceutical value of fruits and vegetables.[26,27] Some studies are evident that prolong microwave heating decreases the content and biological activity of bioactive phytochemical components of food material due to their thermal degradation.[15,28] Therefore, in continuation of the studies on the effect of microwave treatment on the quality of food and pharmaceutical products, a comparative study was designed to investigate the effect of oven heating and microwave treatment methods on the phytochemical and antioxidant potential of ginger.

In the present study, the oven heating and microwave treatment of ginger resulted in a significant decrease in its phytochemical content, total antioxidant activity, and free radical scavenging capacity. Among the two treatment methods, the microwave treatment was found to be comparatively more effective in decreasing the TPA, TFC and DPPH RSC and oven heating showed more effect on TTC, TAOA, and HRSC. However, both treatment methods were found to improve the β-carotene bleaching capacity, reducing power and metal chelating abilities of ginger. The results for TPA, TFC, DPPH RSC and HRSC of untreated ginger rhizome were found to be comparatively higher than those reported earlier in dried ginger.[29] The decrease in the phenolic content and loss of antioxidant potential of an oven heated and microwave treated ginger was in agreement to the studies reported earlier.[15] The decrease in DPPH RSC of ginger in response to an increase in heating time is also in agreement with the previous study.[16] The increase in metal reducing power and the chelating ability of oven heated and microwave treated ginger do not support the results previously reported in other spices subjected to thermal treatment.[15]

The decrease in phytochemical content in response to an increase in the treatment time in both cases may be attributed to the thermal degradation of the heat-sensitive bioactive phytochemical compounds present in

ginger. Prolonged oven heating and thermal treatment at high temperature may cause the structural changes due to thermal degradation of phytochemical constituents resulting in a decrease in their extraction yield in a extracting solvent. It may also decrease the bioavailability and antioxidant potential of these constituents as a part of food products.[15] Microwave heating uses electromagnetic radiation possessing both electric and magnetic fields. The electric field makes the molecules of the sample to vibrate or oscillate due to dipole moment and ionic induction which results in the fast heating of sample.[30] Prolonged heating in this field may result in the electromagnetic disruption of intermolecular and intramolecular hydrogen bonds causing an alteration in the structure and activity of bioactive molecules. The structural changes in the antioxidant phytochemicals such as conjugated polyphenols, phenolic acids, flavonoids, tannins and some vitamins may influence the antioxidant potential of these compounds. The increase in β-carotene bleaching capacity, metal reduction, and metal chelating activity may also be attributed to microwave induced structural changes in phytochemical compounds of ginger.

CONCLUSION

Both heating treatment methods were found to have a significant effect on the phytochemical composition and antioxidant activity of ginger. The heating treatments resulted in a time-dependent decrease in phytochemical composition, total antioxidant activity, and free radical scavenging activities. However, the effect of microwave treatment was found to be comparatively more significant on TPA, TFC, TAOA, ICA and DPPH RSC of ginger. This study provides useful information regarding the potential benefits of ginger and effect of various heat treatments and processing methods on its medicinal value. The study may also be helpful to the manufacturers and consumers in selection of a suitable heating method for ginger based pharmaceutical extraction and cooking of food recipes containing ginger as an ingredient.

ACKNOWLEDGEMENT

The authors are grateful to Institute of Chemical Sciences, Bahauddin Zakariya University, Multan, Pakistan for providing the laboratory facilities throughout the experimental work.

CONFLICT OF INTEREST

There is no conflict of interest regarding this study.

ABBREVIATIONS

TPA: Total phenolic acids; **TTC:** Total tannins content; **TFC:** Total flavonoids content; **TAOA:** Total antioxidant activity; **BCBC:** β-carotene bleaching activity; **RP:** Reducing power; **ICA:** Iron chelating activity; **DPPPH RSC:** 2, 2-diphenyl 1-picrylhydrazyl radical scavenging capacity; **HRSC:** hydroxyl radical scavenging capacity.

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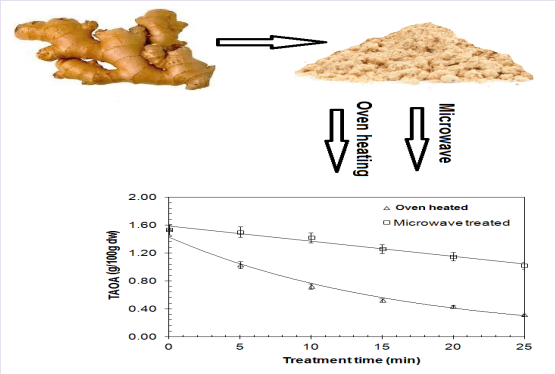
GRAPHICAL ABSTRACT



SUMMARY

- Effect of roasting and microwave treatment was found to be significant on studied parameters. Both of the heating methods showed negative effect on phytochemical content and antioxidant activity. Both of the heating methods showed positive effect on metal reducing, chelating and β-carotene bleaching activity. Microwave treatment showed more significant effect on phytochemical content and antioxidant activity.

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Cite this article: Nawaz H, Shad MA, Aziz AR, Muntaha ST, Muzaffar S. Phytochemical Composition and Antioxidant Potential of Oven Heated and Microwave Treated Ginger (*Zingiber officinale* Roscoe). Free Radicals and Antioxidants. 2018;8(2):89-5.