



## Effect of cooking duration on the phenolic content and antioxidant properties of Black cumin (*Nigella sativa* L)

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**ABSTRACT:** Black cumin (*Nigella sativa* L.) seeds are normally used as spices in food preparations and in folklore for the treatment and management of several diseases. This present study sought to assess the effect of cooking duration (5 – 15 min) on the phenolic content and antioxidant properties of black cumin seed. The results of the study revealed that cooking for 5 – 10 min caused significant ( $P < 0.05$ ) increase in the phenolic content (total phenol and flavonoid) with a significant decrease ( $P < 0.05$ ) at 15 min of cooking. This same pattern was observed for the antioxidant properties of the seed. Hence, minimal heat treatment duration (5 – 10 min) through cooking is recommended to prevent the major loss of antioxidant properties and phenolic constituents of black cumin seed.

**Keywords:** Black cumin seed (*Nigella sativa*); phenolics; antioxidant; cooking; reducing power

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### INTRODUCTION

Black cumin (*Nigella sativa* L.) is an annual flowering plant belonging to *Ranunculaceae* family, widely distributed in countries bordering the Mediterranean Sea, Central Europe and Western Asia (Hashem and El-Kiey, 1982). The seeds are black and triangular in shape with a pungent bitter taste and a faint smell of strawberries. Cumin is one of the commonly used spices in food preparations. Traditionally Cumin is used in medicine as a stimulant, a carminative, an astringent and as remedy against indigestion, flatulence and diarrhoea (Norman, 1990). Black cumin is widely used as spice/condiments in vegetarian and non-vegetarian preparations along with other spices in India and Arabia (Thippeswamy and Naidu, 2005). The seed can be powdered and applied to food preparations during or after cooking.

Thymoquinone, dithymoquinone, thymohydroquinone and thymol are the pharmacologically active quinones of black cumin oil (Ghosheh *et al.*, 1999). Like other spices, cumin has been reported as good source of phenolic phytochemicals with strong antioxidant activity (Thippeswamy and Naidu, 2005). Phytochemicals from spices, are very good antioxidants which offer protection against lipid peroxidation. Antioxidant activities of many plant foods have been found to be a function of the quantity and quality of phenolic phytochemicals present (Chu *et al.*, 2002). The antioxidant properties of phenolics are related to their chemical structure which makes them capable of neutralizing reactive oxygen species, scavenge free radicals, chelate metal catalyst and act to protect the cell. Free radicals and reactive

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oxygen species (ROS) are entire class of highly reactive molecules capable of reacting with almost every known molecule in the biological system in their vicinity. Free radicals damage proteins, cause breakdown of DNA strands and initiate the peroxidation of various compounds. Almost all the vital components of cells are susceptible to damage by free radicals. Free radicals also causes lipid peroxidation which is an autocatalytic mechanism leading to oxidative destruction of cellular membranes (Cheeseman and Slater, 1993). Although extensive studies have been conducted on pharmacological

properties of the essential oil of black cumin and thymoquinone such as; antioxidant activity (Burits and Bucar, 2000), antihypertensive effect (Zaoui *et al.*, 2000) and hepatoprotective properties (Mansour *et al.*, 2001); nevertheless there is still dearth of information on the effect of duration of cooking on the antioxidant properties of black cumin seed. Also, since spices are used as water-extracted paste or dry powder in food preparations (cooked and uncooked), this work was designed to further investigate the effect of cooking on the phenolic content and antioxidant properties of black cumin seed.

## MATERIALS AND METHODS

### Materials

Black cumin seeds (*Nigella sativa* L) were sourced locally from the central market in Kano, Nigeria. The identification and authentication was done at the Department of Biology, Federal University of Technology, Akure, Nigeria. All the chemicals used were of analytical grade, while distilled water was used.

### Methods

#### Sample preparation and aqueous extraction

The seeds were hand-picked to remove dirt and inedible portions. The edible portions were rinsed, sun dried and ground into powder. One gramme of the powdered raw black cumin seed was soaked overnight in 100 ml distilled water. The supernatant was collected and treated as reported in this work. For the cooked samples; 1g of the powdered raw black cumin seed was cooked in 100 ml distilled water in a conical flask with stopper for 5, 10 and 15 min respectively. Thereafter the mixtures were centrifuged at 358g for 10 min and supernatants collected were made up to 100 ml, and later used for further analysis.

#### Determination of total phenol content

The total phenol content was determined on the extracts using the method reported by Singleton *et al.* (1999). Appropriate dilution of the extracts (100  $\mu$ l) were oxidised with 2.5 ml of 10% Folin-Ciocalteu's reagent (v/v) and neutralised with 2.0 ml of 7.5 % sodium carbonate. The reaction mixture was incubated for 40 min at 45 °C and the absorbance was measured at 765 nm (UV – Spectrophotometer JENWAY 6305). The total phenol content was subsequently calculated as Gallic acid equivalent.

#### Determination of total flavonoid content

The total flavonoid content of the extracts were determined using a slightly modified method reported by Meda *et al.* (2005). Briefly, 0.5 ml of appropriately diluted sample was mixed with 0.5 ml methanol, 50  $\mu$ l of 10 % AlCl<sub>3</sub>, 50  $\mu$ l of 1 M potassium acetate and 1.4 ml water. The mixture was allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was

subsequently measured at 415 nm (UV – Spectrophotometer JENWAY 6305). The total flavonoid content was calculated as Quercetin equivalent.

#### **Fe<sup>2+</sup> chelation assay**

The Fe<sup>2+</sup> chelating ability of both extracts were determined using a modified method of Minotti and Aust (1987) with slight modification. Freshly prepared 250  $\mu$ l FeSO<sub>4</sub> (150  $\mu$ l) was added to a reaction mixture containing 168  $\mu$ l of 0.1 M Tris-HCl (pH 7.4), 218  $\mu$ l saline and the extracts (0 – 25  $\mu$ l). The reaction mixture was incubated for 5 min, before the addition of 13  $\mu$ l of 0.25 % 1,10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm (UV – Spectrophotometer JENWAY 6305). Percentage Fe<sup>2+</sup> chelating ability was subsequently calculated.

#### **•OH radical scavenging ability**

The ability of the extracts to prevent Fe<sup>2+</sup>/H<sub>2</sub>O<sub>2</sub>-induced decomposition of deoxyribose was carried out using the method of Halliwell and Gutteridge (1981). Briefly, freshly prepared aqueous extract (0 – 100  $\mu$ l) was added to a reaction mixture containing 120  $\mu$ l 20 mM deoxyribose, 400  $\mu$ l 0.1 M phosphate buffer, 40  $\mu$ l 20 mM hydrogen peroxide and 40  $\mu$ l 500  $\mu$ M FeSO<sub>4</sub> and the volume was made up to 800  $\mu$ l with distilled water. The reaction mixture was incubated at 37 °C for 30 min and the reaction was then stopped by the addition of 0.5 ml of 2.8% trichloroacetic acid (TCA), this was followed by the addition of 0.4 ml of 0.6% thiobarbituric acid (TBA) solution. The tubes were subsequently incubated in boiling water for 20 min. The absorbance was read at 532 nm (UV – Spectrophotometer JENWAY 6305). Percentage  $\dot{Y}$ OH scavenging ability was subsequently calculated.

#### **2,2-azinobis(3-ethylbenzo-thiazoline-6-sulfonate) ABTS<sup>•+</sup> scavenging ability**

The ABTS<sup>•+</sup> scavenging ability of the extracts was determined according to the method described by Re *et al.*, (1999). ABTS<sup>•+</sup> was generated by reacting an ABTS aqueous solution (7 mM) with K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (2.45 mM, final concentration) in the dark for 16 h and adjusting the Absorbance at 734 nm to 0.700 with ethanol. Appropriate dilution (0.2 ml) of the extract was added to 2.0 ml ABTS<sup>•+</sup> solution and the absorbance were read at 734 nm (UV – Spectrophotometer JENWAY 6305) after 15 min. Trolox was used as the standard and subsequently, total antioxidant capacity was calculated as trolox equivalent (TEAC).

#### **Determination of reducing power**

The reducing property of the extracts was determined by assessing the ability of the extract to reduce FeCl<sub>3</sub> solution as described by Oyaizu (1986). A 2.5 ml aliquot was mixed with 2.5 ml of 200 mM 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 was added. This mixture was centrifuged at 358 g for 10 min. Five milliliter of the supernatant was mixed with an equal volume of water and 1 ml of 0.1% ferric chloride was added. The absorbance was read at 700 nm (UV-Spectrophotometer JENWAY 6305). Ascorbic acid was used as standard reducing agent and ferric reducing power (AAE) was subsequently calculated as ascorbic acid equivalent.

#### **Data analysis**

The results of the three replicate experiments were pooled and expressed as mean  $\pm$  standard deviation. One-way analysis of variance (ANOVA) was carried out and significance was accepted at  $P < 0.05$ .

## RESULTS AND DISCUSSIONS

Black cumin seed is a popular spice used as flavour enhancer and in folk medicine for the management of several degenerative diseases. Its role in human nutrition and health could be because of its biological active phytochemicals that may have antioxidant activities. The total extractable phenolic compound of black cumin seed is presented in table 1. The values ranged from 205.81 mg/100g (raw extract) to 240.81 mg/100g (10 min cooked extract). The total phenolic content of the black cumin seed increased in the order raw < 5 min cooked < 10 min cooked. This is an indication that cooking caused a significant increase in the total phenol content of black cumin seed and it is in agreement with the report of Oboh (2005) where increase in the phenolic content of vegetables were observed after blanching. However, there was a significant decrease in the total phenol content of the seed after 10 minutes of cooking (15 min cooked). This finding is consistent with that of Amin *et al.* (2006) on phenolic content of raw and blanched *Amaranthus* specie. Nevertheless, the values recorded are lower than that of aqueous extracts of cumin and bitter cumin (Thippeswamy and Naidu, 2005).

Also, the total flavonoid content of the seed (Table 1) revealed that cooking caused a

significant increase in the flavonoid content from 10.96 mg/100g (raw extract) to 36.47 mg/100g (10 min cooked extract) and a significant ( $P<0.05$ ) decrease (21.23 mg/100g) after 10 minutes of cooking (15 min cooked). The pattern observed for the total flavonoid content of the seed is in agreement with that of total phenolic content where significant ( $P<0.05$ ) decrease was observed after 10 minutes of cooking. Food processing techniques have been reported to influence phenolic content of plant foods (Oboh, 2005). Ismail *et al.* (2004) reported that heat treatment caused a marked reduction in the total phenolic contents of some vegetables. Phenolic compounds have been shown to be responsible for the antioxidant activity of plant materials (Rice-Evans *et al.*, 1996).

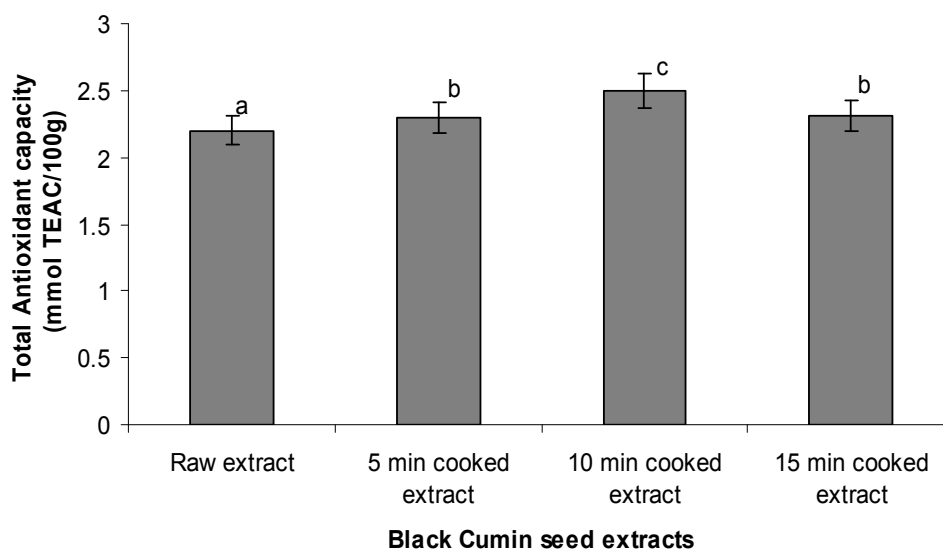
Prevention of the chain initiation step by scavenging various reactive species such as free radicals is considered an important antioxidant mode of action (Dastmalchi *et al.*, 2007). Therefore, the free radical scavenging ability of the black cumin extracts was studied using a moderately stable nitrogen-centered radical species; ABTS<sup>•+</sup> (Re *et al.*, 1999). The ABTS radical based model of free radical scavenging ability has the advantage of being more versatile

**Table 1: Effect of cooking time (5 – 15 min) on the total phenol and total flavonoid content of black cumin seed**

Time period	Raw	5 min cooked	10 min cooked	15 min cooked
Total phenol (mg/100g)	205.81 <sup>d</sup> ±7.31	220.31 <sup>b</sup> ±4.95	240.81 <sup>a</sup> ±8.00	216.74 <sup>c</sup> ±6.50
Total flavonoid (mg/100g)	10.96 <sup>d</sup> ±0.48	32.88 <sup>b</sup> ±1.45	36.47 <sup>a</sup> ±0.73	21.23 <sup>c</sup> ±1.40

Values represent mean ± standard deviation of triplicate experiments

Values with the same superscript letters along the same column are not significantly ( $P<0.05$ ) different.

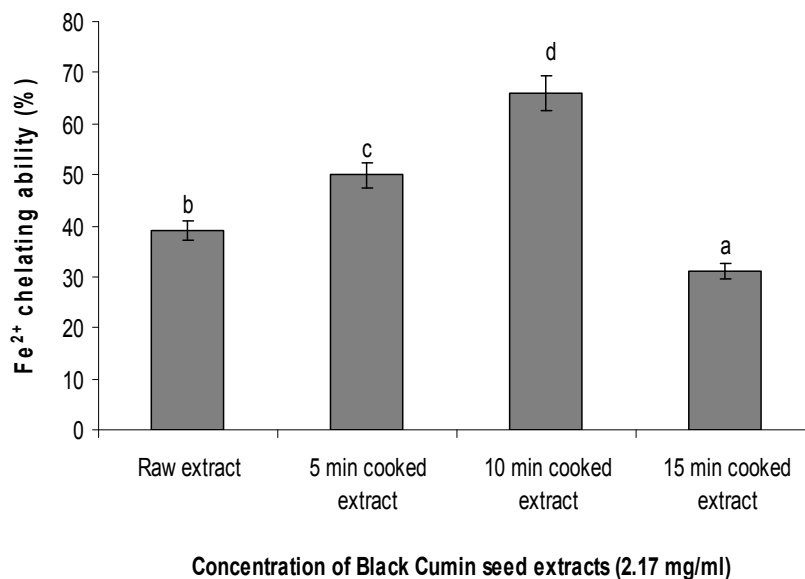


**Figure 1: Effect of cooking time (5 – 15 min) on the total antioxidant capacity of Black Cumin seed**

due to minimal spectral interference as the absorption maximum used is 760 nm, a wavelength not normally encountered with natural products (Re *et al.*, 1999). ABTS<sup>+</sup> scavenging ability reported as TROLOX equivalent antioxidant capacity (TEAC) is presented in Figure 1. The result revealed that cooking for 5 and 10 min caused no significant increase in the radical scavenging ability (2.29 and 2.24 mmol/100g respectively) when compared with the raw seed (2.16 mmol/100g). However, a significant ( $P < 0.05$ ) decrease in the radical scavenging ability was observed after 15 min. The trend in the ABTS<sup>+</sup> scavenging ability of extracts also agreed with the phenolic contents. Findings have shown that total antioxidant activity of plant foods is a function of their phenolic content, hence, the decrease in the ABTS<sup>+</sup> scavenging ability of the extract after cooking for 10 minutes suggests reduction in the phenolic contents.

The ability to chelate and deactivate transition metals, prevent such metals from participating in the initiation of lipid peroxidation and

oxidative stress through metal catalysed reaction is considered one of the mechanism through which phenolics exhibit their antioxidant activity (Oboh *et al.*, 2007). The Fe<sup>2+</sup> chelating ability (Figure 2) of the black cumin seed extracts increased from 38.07% (raw) to 60.65% (10 min cooked) at the concentration tested (2.17 mg/ml), however at 15 min, a significant ( $P < 0.05$ ) decrease in the Fe<sup>2+</sup> chelating ability was observed. This is in agreement with the phenolic content (total phenol and flavonoid) of the black cumin seed as influenced by the cooking time. Findings have shown that antioxidant capacity of plant food is a function of their phenolic content (Chu *et al.*, 2002), hence, the observed decrease in the Fe<sup>2+</sup> chelating ability of the black seed extracts cooked at 15 minutes of cooking. Nevertheless, the Fe<sup>2+</sup> chelating ability of the extracts fall within that reported for hot pepper (Oboh *et al.*, 2007). Iron, an essential metal for normal cellular physiology, had been implicated in cellular injury (when in excess), because it plays a catalytic role in the initiation of free radical reactions. The mechanism by which iron

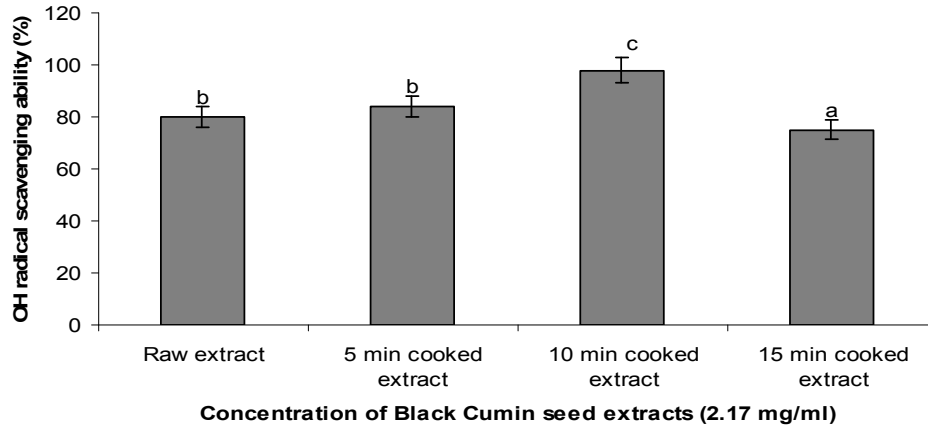


**Figure 2: Effect of cooking time (5 – 15 min) on the Fe<sup>2+</sup> chelating ability of Black Cumin seed**

can cause this deleterious effect is that Fe<sup>2+</sup> can react with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) to produce the hydroxyl radical (<sup>•</sup>OH) via the Fenton reaction (Fraga and Oteiza, 2002). Oboh et al. (2007) reported that Fe<sup>2+</sup> chelation may be part of the mechanism by which plant polyphenols prevents Fe<sup>2+</sup> – induced lipid peroxidation in tissues and oxidative stress mediated by iron overloads. The high Fe<sup>2+</sup> – chelating ability of black cumin could be of immense importance in the protection against oxidative stress, because it is usually too late to attempt to use <sup>•</sup>OH scavengers for therapeutic purposes, due to extreme high reactivity of hydroxyl radicals towards most biomolecules and would require unreasonably high concentrations of intercepting scavengers to neutralize the biomolecules of interest. These very high concentrations of scavengers are difficult to achieve *in vivo*. Consequently, they are not likely to be used for therapeutic purposes,

thereby making Fe<sup>2+</sup> chelators a better therapeutic alternative (Bayir *et al.*, 2006).

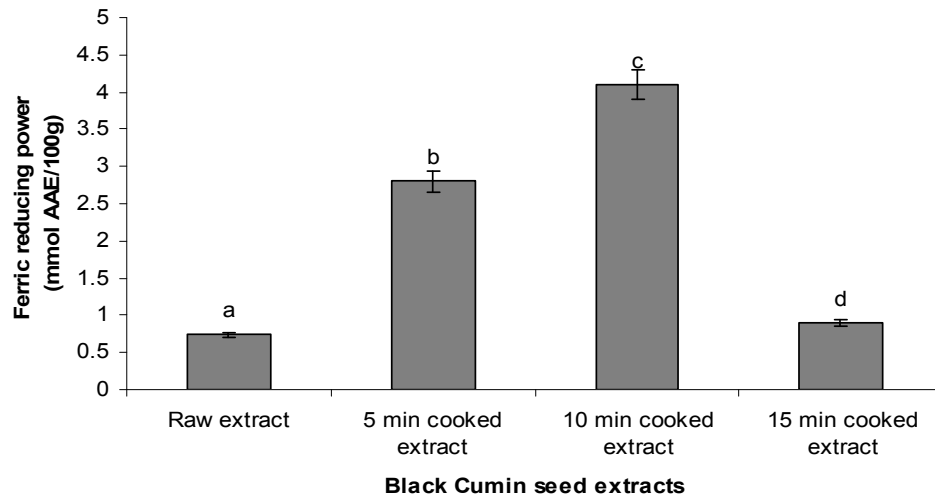
The <sup>•</sup>OH scavenging ability of the black cumin seed extracts is as presented in Figure 3. The results revealed that cooking for both 5 and 10 min caused a significant ( $P < 0.05$ ) increase in <sup>•</sup>OH scavenging ability of the seed ranging from 78.24% to 95.34% respectively, with significant ( $P < 0.05$ ) reduction at 15 min of cooking. This also agreed with the phenolic content of the seed as affected by cooking. Nevertheless, black cumin seed extracts has a stronger <sup>•</sup>OH scavenging ability compared with what was reported for tree pepper (Oboh and Rocha, 2008), and red and green hot pepper (Oboh *et al.*, 2007). <sup>•</sup>OH is a highly reactive ROS usually produced as a result of Fe catalysed reaction (Fenton) (Fraga and Oteiza, 2002). It is capable of attacking most biomolecules e.g.; carbohydrates, DNA, polyunsaturated fatty acids and proteins. The prevention of such deleterious reactions is highly significant in human health.



**Figure 3: Effect of cooking time (5 – 15 min) on the  $\cdot$ OH scavenging ability of Black Cumin seed**

As represented in Figure 4, the reducing power of the black cumin seed extracts as ascorbic acid equivalent (AAE) increased from 0.74 mmol.AAE/100g (raw) to 4.11 mmol.AAE/100g (10 min boiled). However, a significant decrease in the reducing power was observed at 15 min of cooking (0.89 mmol.AAE/100g) and the trend of the reducing power also followed the pattern of the phenolic content where cooking (5 and 10 min) caused significant increase ( $P < 0.05$ ) and decrease at 15 min of cooking time. Reducing power is a potent antioxidant defense mechanism, which explores the electron and/or hydrogen atom transfer potential of antioxidant molecules. This is based on the ability to reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$ , because, the ferric-to-ferrous iron reduction occurs rapidly with all reductants with half reaction reduction potentials above that of  $\text{Fe}^{3+}/\text{Fe}^{2+}$ ; the values in the ferric reducing antioxidant properties (FRAP) assay will express the corresponding concentration of electron-donating antioxidants (Halvorsen *et al.*, 2002).

The reducing power of the extracts may be due to their phytochemical constituents. Antioxidant activity of phenolic compounds have been linked to its redox properties which allowed them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Rice-Evans *et al.*, 1996). Antioxidant activities of plant food have been shown to correlate with the phenolic content of the food (Chu *et al.*, 2002). Any process or activities that lead to the reduction of the phenolic content of the food may have a reducing effect on the total antioxidant properties of the food. Different cooking times might have resulted in some loss of antioxidants such as ascorbic acid,  $\alpha$ -tocopherol and phenolic compounds such as flavonoids. On the other hand, heat also tends to degrade certain compounds with antioxidant properties (Amin and Lee, 2005). The loss in the antioxidant capacity of black cumin seed may be due to losses or degradation of certain types of phenolic compounds or other free radical-scavenger components after cooking for more than 10 minutes.



**Figure 4: Effect of cooking time (5 – 15 min) on the Reducing power of Black Cumin seed**

## CONCLUSION

Cooking for few minutes (5 – 10 min) considerably increased the phenolic content (total phenol and total flavonoid content) and antioxidant properties of black cumin seed. However, cooking for 15 min caused a negative effect on the phenolic content and a reduction in the antioxi-

dant properties. Thus, minimal heat treatment duration (5 – 10 min) through cooking is recommended to prevent the major loss of antioxidant properties and phenolic content of black cumin seed.

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