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Effect of Conventional and Superheated Steam Roasting on the Total Phenolic Content, Total Flavonoid Content and DPPH Radical Scavenging Activities of Black Cumin Seeds

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ABSTRACT

The effect of superheated steam heating on the antioxidant properties of black cumin seed (*Nigella sativa* L.) compared to conventional hot air heating was investigated. A superheated steam oven was used in both methods using superheated steam mode and convection mode (hot air). It was operating at three different durations of 10, 20 and 30 min at 180 °C. The total phenolic content, total flavonoid content and radical scavenging activities were 15.04 mg GAE /g, 0.81 mg QE /g and 81.28% at 180°C for 30 min, respectively during superheated steaming. The total phenolic content (TPC), total flavonoid content (TFC) and DPPH radical-scavenging activities of black cumin seed increased significantly ($p<0.05$) when the time of heating was increased from 0 to 30 min for both treatments. The raw seed had the lowest antioxidant properties, which were TPC of 5.17 mg (GAE)/g, TFC of 0.29 mg (QE)/g and 61.27% radical scavenging activities. Positive correlations were found between the total phenolic content and DPPH scavenging activities of black cumin seed. The black cumin seed heated under superheated steam had significantly ($p<0.05$) higher TPC, TFC and DPPH radical scavenging activities compared with conventional hot air heating at almost all the heating times. Based on the results obtained, it can be concluded that superheated steam can be used as an alternative method for roasting black cumin seed that yet maintains higher antioxidant properties.

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INTRODUCTION

Black cumin (*Nigella sativa* Linn.) seed, which belongs to the family Ranunculaceae, is a type of spice native to the Mediterranean region (Lutterodt et al., 2010). One of the most useful parts of the plant is the seeds, which are utilised worldwide for edible and medicinal applications (Ramadan, 2007). The seeds of *Nigella sativa* are widely utilised for medicines traditionally in India, Pakistan, Saudi Arabia, China and some other countries for the cure of headache, bronchitis, fever, rheumatism, liver and kidney disorders, influenza, cough and asthma and as a diuretic. The extract from the seeds, containing thymoquinone, has been reported as an active antioxidant. Spices are functional ingredients in food products. One of their major functions in the food industry is to enhance the taste of food. Spices also act as a protective, antioxidant and antimicrobial agent in human health (Nagi & Mansour, 2000; Lee et al., 2004; Dawidowicz et al., 2006).

Black cumin seed is a spice that is widely used in Indian cuisine due to its aromatic nature. It is used as a spice in the Indian sub-continent for various dishes, especially those that require a preservative. India is the biggest producer of these seeds in the world. Nepal, Sri Lanka, Bangladesh, Pakistan, Egypt and Iraq also produce black cumin seeds. Recent scientific investigation reported that the seeds show potential medicinal value including anti-carcinogenic, antibacterial, analgesic, anti-inflammatory, antipyretic and antiulcer properties. The exceptional curative activities of the seed can be ascribed to its phenolic components that comprise the highest levels of antioxidant properties (Nagi & Mansour, 2000; Lee et al., 2004). There is growing indication that intake of a selection of phenolic components existing in natural spice-enhanced foods may reduce the threat of chronic disease due to the antioxidant properties of these products. Black cumin seeds have a strong spicy, warm, heavy, curry-like and hot, peppery taste. These features are dominated by cumin aldehyde. (Dawidowicz et al., 2006; Kanakdande et al., 2006).

The seeds have also been reported to be used in bakery and confectionery products. The seeds are sprinkled on loaves during bread making (Kiralan, 2012). A conventional hot air oven is regularly used for baking bread at a selected temperature and time of 180°C for 17 min. Black cumin seeds are also often dry roasted under hot air prior to their use as a culinary ingredient. However, a limitation of using a conventional hot air oven is that the heat transfer for baking using this method reduces the seeds' antioxidant properties and phenolic compounds due to oxidation (Zzaman et al., 2014).

Superheated steam is an emerging technology that can be produced by heating saturated steam at a temperature higher than that of the boiling point of water. There are many inherent properties of superheated steam that make it attractive for not only drying but for many processing applications as well (Pronyk et al., 2004; Shan et al., 2016). The thermal properties

of steam are higher than those of air at the same temperature, resulting in a higher heat transfer coefficient. Superheated steam provides an oxygen-free environment that may improve product qualities and eliminate fire and explosion hazards. Superheated steam has been successfully applied to many types of food product, including potato chips, tortilla chips, instant noodles, shrimp and others (Li et al., 1999). Most of the previous investigation into black cumin seeds showed that they have a potential activity as an antioxidant. However, the impact of superheated steaming and conventional hot air roasting on the antioxidant activities of black cumin seed has not been investigated. Therefore, the aims of this research were to evaluate the impact of superheated steam and conventional hot air roasting at different time spans on the total phenolic, total flavonoid and radical scavenging activities of black cumin (*N. Sativa*) seeds.

MATERIALS AND METHOD

Raw Material

Black cumin (*Nigella Sativa* L.) seeds imported from India were purchased from a local supermarket located at Bukit Jambul, Penang. The moisture content was less than 2% of the weight of the seeds. The seeds were stored in a hermetic container at room temperature until further use.

Chemicals

Gallic acid and aluminum chloride were obtained from Fisher Scientific, UK. Methanol (99.8%) and sodium carbonate were obtained from QReC, New Zealand. Folin-Ciocalteu reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Merck (Darmstadt, Germany). Potassium acetate was obtained from UNILAB, the Philippines. Quercetin was obtained from Sigma-Aldrich (USA). All chemicals and reagents used were analytical grades.

Roasting of Black cumin seed

The collected black cumin seeds were allowed to equilibrate before roasting at room temperature overnight. Approximately 100 g portions of cleaned medium size mature black seeds were used for roasting in this study. A superheated steam oven (SHARP, AX-1500) were used for the roasting of the seeds. The roasting process was carried out at 180°C for a duration of 10, 20 and 30 min.

Sample Extract Preparation

After roasting, the roasted and raw seeds were ground in a coffee blender at high speed for 4 min (Lebensstil Kollektion, Germany). The ground seeds were passed through a 1000 micron sieve and then stored in a plastic container until the samples were analysed. Prior to the extraction, the seeds were defatted by a Soxhlet apparatus at 80°C using petroleum ether (AOAC, 2000). The resulting defatted powder was dried at 60°C for 16 to 18 h. The dried and defatted powder samples were stored in a hermetic bottle; this was followed by

extraction, where 0.5 g of each dried sample was prepared for extraction with 20 mL of solvent following the method described by Bucić-Kojić et al. (2011). The solvent was prepared by mixing 99.8% methanol and water to obtain 80% (v/v) aqueous methanol. The extraction was fixed in a Waterbath (90°C) shaker (Schwabach, Germany) and shaken for 120 minutes at 200 rpm. The suspension was centrifuged then for 25 min at 2300 x g (Kubota Tabletop Centrifuge Model 4000, Japan). The supernatant was separated to obtain the methanolic extract using **disposable Pasteur pipets**. The extracts were used in further experiments.

Total Phenolic Content

The total phenolic content of black cumin seed extract was measured based on the Folin-Ciocalteu assay according to the method described by Shin et al. (2014), with slight modification. Briefly, 40 µL of the black cumin seed extract was diluted by 3160 µL of distilled water, followed by addition of 200 µL of the Folin-Ciocalteu reagent, then allowed to react for 5 min. After this, 600 µL of 20% sodium carbonate solution was added to the reaction mixture. The solution was incubated at room temperature for 60 min. After incubation, the absorbance was read at 765 nm against a blank reagent without sample using a UV-Vis 1240 spectrophotometer (Shimadzu Corp, Nagakyo-ku, Kyoto, Japan). The analyses were performed in triplicate. Gallic acid was used as the standard in the calibration curve preparation (40-320 mg/L). The final results were expressed as mg of gallic acid equivalent per gram of seed weight.

Total Flavonoid Content

The aluminum chloride colorimetric method was used for total flavonoid determination according to the method described by Chen and Kitts (2008), with slight modification. Quercetin was used as the standard in the calibration curve preparation. The standard was prepared by dissolving 10 mg of quercetin in 100 ml methanol and then diluted to 10, 20, 30, 40, 50, 60, 100 mg/L using methanol. Briefly, 0.5 ml of seed extract was separately added into test tubes and mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The tubes were covered with parafilm and incubated at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm. The analyses were performed in triplicate. The flavonoid content in each extract was expressed as mg quercetin equivalent (QE)/g of black cumin seed.

DPPH Radical Scavenging Activities

The free radical scavenging activities of black cumin seed samples were estimated according to the method of Sánchez-Moreno et al. (1998), with some modification. The analysis was performed based on the activities of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The DPPH

is a commercially available free radical that is soluble and stable in methanol. In brief, 0.2 mL of the extract was added to 3.8 mL of DPPH solution (0.1 mM) in methanol. The mixture was left to incubate for 30 min at room temperature in a dark place. After incubation, the absorbance of the sample and control (DPPH without sample extract) was read at 517 nm. The analyses were performed in triplicate. The scavenging activity was determined based on the percentage of DPPH radical scavenging activities. The percentage of DPPH radical inhibition was measured using the following equation:

$$\% \text{ DPPH free radical scavenging activities} = [(Ac517 - As517)/(Ac517)] \times 100\%$$

where, Ac517 is the absorbance of control at 517nm and As517 is the absorbance of the sample at 517 nm.

Statistical Analysis

The data obtained were presented as means ± standard deviation (SD) and differences between both treatments were determined using a paired t-test. All measurements were performed in triplicate and the difference between each condition of treatment was analysed with the one-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) 22.0. The significant difference was considered at the level of $p<0.05$.

RESULTS AND DISCUSSION

Analysis of Total Phenolic Content (TPC)

The implemented one-way ANOVA indicated that time significantly ($p<0.05$) affected the total phenolic content, total flavonoid content and % DPPH scavenging activities of black cumin seed after treatment using both conventional hot air and superheated steam drying as shown in Figures 1, 2 and 3.

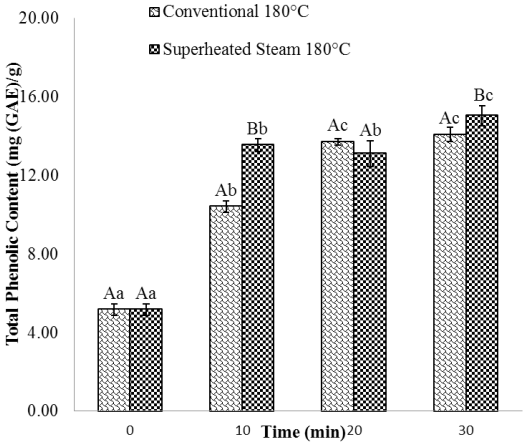

Figure 1. Changes in the total phenolic content of black cumin seed subjected to conventional hot air and superheated steam roasting at different time spans (0-30 min). Each analysis was performed in triplicate. Bars labelled with different capital letters (A and B) within the same time are significantly different at $p<0.05$. Bars labelled with different small letters (a through c) within the same treatment are significantly different at $p<0.05$

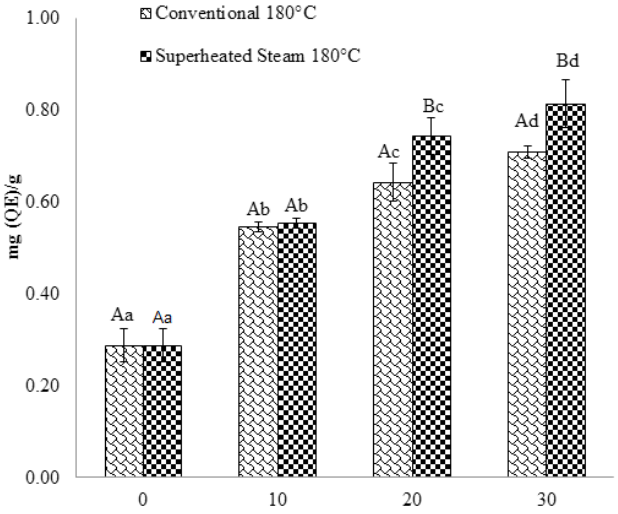

Figure 2. Changes in the total flavonoid content of black cumin seed subjected to conventional hot air and superheated steam roasting at different time spans (0-30 min). Each analysis was performed in triplicate. Bars labelled with different capital letters (A and B) within the same time are significantly different at $p<0.05$. Bars labelled with different small letters (a through d) within the same treatment are significantly different at $p<0.05$

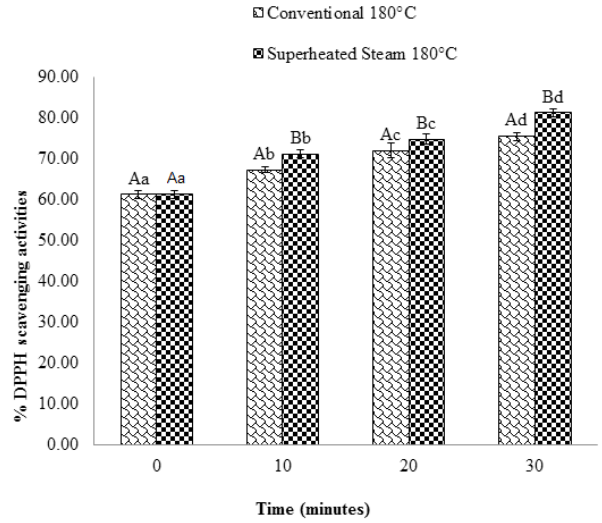

Figure 3. Changes in the DPPH radical scavenging activities of black cumin seed subjected to conventional hot air and superheated steam roasting at different time spans (0-30 min). Each analysis was performed in triplicate. Bars labelled with different capital letters (A and B) within the same time are significantly different at $p<0.05$. Bars labelled with different small letters (a through d) within the same treatment are significantly different at $p<0.05$

Generally, the total phenolic content increased significantly ($p<0.05$) for both treatments, with conventional hot air and superheated steam roasting with the increase in time. At the early stage of heating to 10 min, the total phenolic content was increased twice and 2.5 times when roasted using both conventional and superheated steam that was increased from 5.17 to 10.41 and 13.55 mg GAE/g, respectively. The total phenolic content was increased further when the heating time increased to 30 min. It seemed that the longer time of heating of black cumin seeds broke the covalently bound phenolic compound on the cell wall to release the phenolic compound, which was readily soluble in methanol. It is believed that this phenolic compound may be beneficial for its antioxidant properties (Choi et al., 2006). Another reason for increased antioxidant content at the time of heating was that heating inactivates the endogenous oxidative enzyme, preventing further oxidation of antioxidant compounds in the raw plant material (Nicoli et al., 1999; Dewanto et al., 2002).

Interestingly, the total phenolic content for the superheated steam-roasted sample was also higher compared with that of the conventional hot-air-roasted sample, except for 20 min when no significant difference was detected. However, during the initial stage of the superheated steaming, the total phenolic content also increased 2.5-fold from 5.17 to 13.55 mg GAE/g compared with conventional hot air heating, which showed a lower increment of 2-fold from 5.17 to 10.41 mg GAE/g. Superheated steam has a high heat transfer coefficient, especially at the initial stage of heating than hot-air drying (Ohishi & Shibukawa, 2010). The higher heat transfer coefficient of superheated steam seemed to have more effectively cleft the covalently bound phenolic compound from the seed. As the heating time was increased, the superheated steamed sample also showed a higher total phenolic content than that seen after conventional hot air heating. The highest amount of total phenolic content was found at 180°C after heating for 30 min using superheated steam at 15.04 mg GAE/g. Some studies have suggested that superheated steam-heated foods can retain antioxidants, vitamins and other essential nutrients due to the absence of oxygen, thus reducing oxidation of antioxidant compounds (Head et al., 2010; Wang et al., 2012).

There is no information available in the literature on the effects of superheated steam and conventional hot air heating on the phenolic content of black cumin seed. However, for other plants, the effect of heating treatment on total phenolic content has been reported. Dewanto et al. (2002) reported significantly higher concentrations of the soluble phenolic compound in commercially processed sweet corn compared with fresh ones. They suggested that soluble the phenolic compound in sweet corn can be liberated by heat treatment. Kim et al. (2006) reported that total the phenolic compound in grape seed extract increased when heating time

was increased. Their previous study on sesame seed (Jeong et al., 2004a) and citrus peel (Jeong et al., 2004b) also showed an increase in total phenolic content when thermally treated compared with when non-thermally treated. All their studies concluded that the increment in the total phenolic compound as heating time was increased was because heat treatment can convert an insoluble phenolic compound to a soluble phenolic compound, which can be extracted from a solvent (Jeong et al. 2004a; Kim et al., 2006). Ahmad-Qasem et al. (2013), reporting on the effect of heating of olive pomace on the antioxidant properties of the olive fruit, found that at a higher temperature, thermal processing of olive pomace showed a higher total phenolic content and the total phenolic content increased as the time was increased from 10 min to 30 min. It also found that heating time prolonged from 30 to 60 min did not significantly ($p<0.05$) affect total phenolic content. Wang et al. (2012) reported that sweet potato roasted using superheated steam had a higher total phenolic content than that found in conventional hot-air-roasted sweet potato. The total phenolic content also increased when the heating time was increased to 40 min, but when heating time was more than 40 min, the total phenolic content decreased gradually. Therefore, it is likely that the total phenolic compound of N. Sativa depends on the type of thermal treatment and duration of heating.

Analysis of Total Flavonoid Content (TFC)

Total flavonoid was determined using an aluminum chloride colorimetric assay and the results were expressed in milligrams of quercetin equivalent (QE) per gram of seed. Briefly, the total flavonoid content increased significantly ($p<0.05$) as the heating time was increased regardless of type of thermal treatment. The results suggested that heat treatment might produce changes in extractability due to the disruption to the plant cell wall, thus flavonoid compounds may be released more easily as a result of heat treatment compared with in a raw material (Peleg et al., 1991). The mean total flavonoid content of raw black cumin seed was 0.29 mg (QE)/g. After heating under conventional hot air and superheated steam conditions for 10 min, the total flavonoid content of both treatment had increased to 0.55 mg (QE)/g, which did not show a significant difference between both treatments at that particular time. However, as the heating time was increased to 20 and 30 min, the seeds that were heated using superheated steam showed a higher total flavonoid content than those heated using conventional hot air. The highest total flavonoid content of 0.81 mg (QE)/g was found in the seeds that had been heated at 180 °C under superheated steam for 30 min. Flavonoids such as quercetin are easily oxidised by oxygen. Superheated steam uses water in the form of steam instead of air as the heating medium to minimise oxidation (Head et al., 2001; Wang et al., 2012).

There is as yet no information on the effect of heating treatment on the flavonoid content of black cumin seed. However, the results of this study were consistent with those obtained from studying other plants as reported, for instance, by Choi et al. (2006), who reported significantly higher concentrations of free flavonoid content in Shiittake mushroom compared with that in raw Shiitake mushroom. Wang et al. (2012) reported that sweet potatoes roasted using conventional hot air and superheated steam also showed an increase in total flavonoid content as the heating time was increased to 40 min.

Analysis DPPH Radical Scavenging Activity

Free radicals are reactive species and are known to damage proteins, cause breakdown of DNA strands, initiate peroxidation and trigger various health problems and degenerative diseases such as cancer. Flavonoid and phenolic compounds in plants are the constituents that provide free radical scavenging ability due to their good hydrogen and electron acceptor activities. The DPPH radical scavenging activities assay is one of the known methods to measure antioxidant activities in black cumin seed (Mariod et al., 2009). The DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable radical showing maximum absorbance at 517 nm. Its purple colour fades rapidly when DPPH encounters radical scavengers. The reduction in the DPPH absorbance is the measure of scavenging activities.

The percentage of radical scavenging activities of the seed samples treated with conventional hot air and superheated steam in this study are shown in Figure 3. Generally, DPPH radical scavenging activities increased significantly ($p<0.05$) as heating time was increased in both thermal treatments. After the samples were heated using conventional hot air and superheated steam for 10 min, the DPPH radical scavenging activities increased to 67.30% and 71.20%, respectively. Radical scavenging activities increased further when heating was increased; the highest scavenging activity of 81.28% was found after treatment using superheated steam for 30 min. When the two methods of heating were compared, the results showed that the seeds heated using superheated steam showed higher scavenging activities than those heated using conventional hot air as heating time was increased. The results showed that black cumin seeds heated using superheated steam for 30 min showed the highest scavenging activities for DPPH, whereas the seeds heated using conventional hot air for 10 min showed the lowest. The superheated steam heating system is an oxygen-free environment as water is used as the heating medium, thus less oxidation occurs, resulting in higher antioxidant properties than when the conventional hot air heating system is used because oxygen is absent in the system (Head et al., 2010; Wang et al., 2012). Research into the effects of conventional hot air heating on antioxidant activities of other plant seeds, particularly grape seed,

also showed a significant increase as the heating time was increased (Kim et al., 2006). Rumruaytum et al. (2014) reported that the effect of superheated steam drying at 170°C on the antioxidant activities of a native rice cultivar increased as the heating time was increased because heating can stimulate Maillard reaction and yield (Perez-Jimenez & Saura-Calixco, 2005).

Correlation Analysis of Differences Between the Antioxidant Compounds (Total Phenolic Content) and Antioxidant Activity (DPPH Scavenging Activity)

A correlation analysis studying the differences between the antioxidant compounds (phenolic content) and antioxidant activities (DPPH scavenging activity) of black cumin seed was carried out, and the results are shown in Figures 4 and 5. Based on Figures 4 and 5, a significant positive correlation of R^2=0.95 and R^2=0.86 ($p<0.05$) was found between total phenolic content and DPPH scavenging activities for conventional hot air and superheated steam heating, respectively. Many studies in the literature have presented positive correlations between the total phenolic content and the DPPH free radical scavenging activities (Lim et al., 2007; Mariod et al., 2009). The total flavonoid content of black cumin seed is relatively low, thus it did not contribute much towards the antioxidant activities of black cumin seed. The increase in DPPH radical scavenging activities was due to the increase in the amount of polyphenolic constituents present in black cumin seed that act as free radical scavengers (Choi et al., 2006; Mariod et al., 2009).

Research has not yet reported on the effect of conventional hot air and superheated steam on the antioxidant activities of black cumin seed. However, for other plants, the results reported were consistent with the research performed by Wang et al. (2012), studying the roasting of sweet potatoes, found that the DPPH scavenging activities of the superheated steam sample were higher than those of the conventional hot-air-roasted sample. The DPPH scavenging effect of cooked sweet potatoes was also higher than that of raw sweet potatoes as the increase in DPPH scavenging effect was due to the increase in total phenolic content (Teow et al., 2007). Jeong et al. (2004b) reported that the DPPH scavenging activities of sesame seed increased as the roasting time was increased at 150°C and 200°C, whereas roasting at a lower temperature, particularly at 50°C and 100°C, did not change the radical scavenging activities significantly.

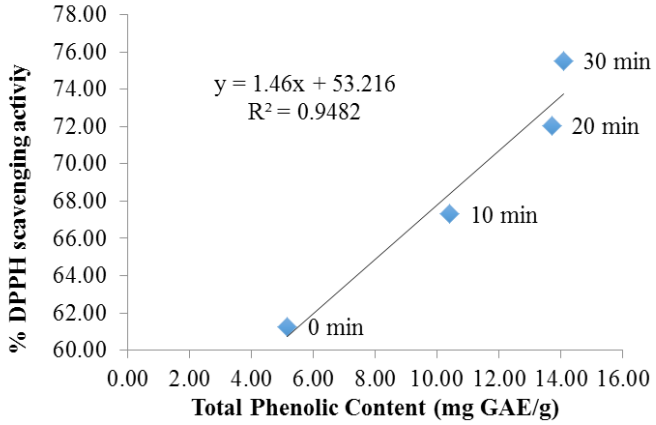

Figure 4. Correlation between DPPH radical scavenging activities and total phenolic content of black cumin seed subjected to conventional hot air heating

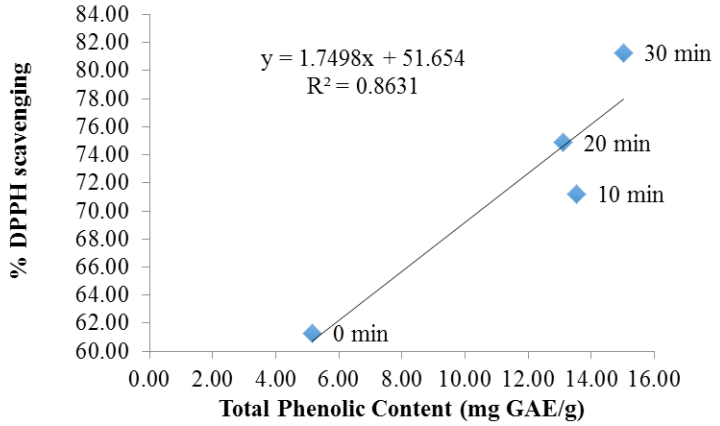

Figure 5. Correlation between DPPH radical scavenging activities and total phenolic content of black cumin seed subjected to superheated steam heating

CONCLUSION

Superheated steam is a low-oxygen heating medium that can prevent antioxidants from being oxidised by minimising oxidation. Therefore, it is concluded that superheated steam heating can maintain the quality of black cumin seed, in addition to enhancing scavenging activities and increasing phenolic and flavonoid content. This study, therefore, supports the use of superheated steam as an alternative heat treatment for black cumin seeds. However, future studies should examine other characteristics such as volatile compounds, colour etc.

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