

OPTIMIZED ROASTING CONDITIONS FOR ENHANCING NUTRACEUTICAL VALUE OF SESAME (*Sesamum indicum* L.) SEEDS

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ABSTRACT

Limited information is available on the characterization of nutraceutical rich Indian sesame genotypes for their antioxidative properties and on the development of protocols to harness the maximum availability of nutraceutical components to consumers. We developed a roasting protocol for harnessing maximum nutraceuticals from sesame seeds. For this purpose, a set of nine genotypes holding high variability in oil content (35.4-50.1%) and seed colour were roasted at temperatures (180°C, 200°C and 220°C) for different time periods (10min, 20min, 30min, 60min). Roasting showed little effect on fatty acid profile. Total phenols, tocopherols, total antioxidant activity and DPPH radical scavenging capacity significantly increased with increase in roasting temperature. The study recommends that roasting temperatures (180°C, 200°C and 220°C) with longer roasting time (20-60 min) resulted in higher antioxidative profile than control with maximum values at 220°C for 20 to 60 min.

Keywords: Antioxidant activity, Fatty acid composition, Phenols, Roasting, Tocopherols

The tiny seeds of sesame are graced with an amazing union of nutritional and medicinal qualities which have raised its value world over owing to which planting area of sesame has almost doubled in the last fifty years. At present, sesame is cultivated in over 70 countries in both tropical and temperate zones of Africa, Asia, Latin America, and some parts of the southern United States. According to FAOSTAT (2021), the top five producers of sesame are Sudan (1.12 MT), India (0.82 MT), Tanzania (0.70 MT), Myanmar (0.64 MT), and China (0.46 MT). The Indian figures are just the rough estimates as sesame is also grown by small farmers and the harvest is consumed locally without any record of trade and domestic processing. Sesamum seeds are exported from India not only in raw form but in various other forms viz., roasted seeds, roasted powder, hulled seeds, oil and paste etc.

Sesame has been cultivated for centuries, for its high content of edible oil (34-63%) and protein (18-25%). The seed colour of sesame seeds varies from white, yellow, grey, red, brown to black. Light colored seeds usually have high oil content compared to dark sesame seeds (Zhang *et al.*, 2013). Sesamum seeds are rich source of total carbohydrates (8.2-11.26%), proteins (23.10-24.43%), minerals (calcium, copper, phosphorus, iron, magnesium, manganese and zinc) along with sufficient quantity of dietary fibre, antioxidants, vitamins B₁ (thiamine) and E (tocopherol) (Aglave, 2018). The oil extracted from sesamum seeds is highly valued for its nutritive quality and exceptional resistance to rancidity. The protective nature of sesame oil towards oxidation

is due to the richness in antioxidants viz, tocopherols (Vitamin E), lignan (0.4-1.1% sesamin, 0.3-0.6% sesamol and traces of sesamol) which imparts long shelf life to oil (Philip *et al.*, 2010). Due to its flavour, stability and extraordinary curative properties, sesame fat has occupied a significant slot in the food industry and hence designated as "Queen of oilseeds".

Sesame oil is preferred over other vegetable oils because it constitutes more than 80% of oleic (MUFA) (35-42%) and linoleic acid (omega6) (PUFA) (41-47%) of the total fatty acids and less than 20% of SFA, mainly palmitic (8-12%) and stearic (5-6%). Thus, sesame seeds are a rich source of an essential fatty acid, omega 6 (linoleic acid) which has a crucial role in brain function, regulating the metabolism of the body, maintaining the reproductive system and stimulating skin and hair growth. Sesame oil is unique compared to other vegetable oils it contains approximately equal proportions of oleic and linoleic acid (Jinyoung *et al.*, 2008). The fatty acid profile of sesame is responsible for lowering bad cholesterol (low-density lipoprotein, LDL) and maintaining good cholesterol (high-density lipoprotein, HDL). Despite having highly unsaturated fatty acid such as linoleic acid (C_{18:2}), sesame oil is remarkably stable to oxidation compared to other vegetable oils because of its richness in antioxidants (Hassanein *et al.*, 2012).

It is well known that conditions of roasting process highly affect the antioxidant factors imparting stability to sesame seeds (Jannat *et al.*, 2010). Therefore, in order to grab more nutraceuticals from these tiny potent sesamum seeds, the optimum roasting conditions should be defined. The objective of the present study

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was to elucidate the effect of roasting temperatures (180°C, 200°C, 220°C) and time (10, 20, 30, 60 min) on fatty acid profile, total phenol content, tocopherols, total antioxidant activity and DPPH radical scavenging capacity of the sesame seeds. The study also aims to establish the nutritional qualities of raw and processed sesame seeds grown in India.

MATERIALS AND METHODS

Sample material and roasting conditions

Nine cultivars of sesame seeds (*Sesamum indicum* L.) (Pb Til No.2, RT 346, LT-16-132-1, LT-16-276-2, AT-338, LT-16-401-1, LT-15-28, LT-16-296-1 and LT-16-374-1) were grown in the summer of 2018 at PAU, Ludhiana. The harvested sesame seeds were cleaned manually and used to study the effect of roasting on quality attributes. The seeds were placed in petri dishes (16 cm diameter) covering with the other petri dish and then roasted in an electric baking oven at temperatures of 180, 200 and 220°C for 10, 20, 30 and 60 min respectively. During roasting, seeds were periodically stirred. After each treatment, seeds were allowed to cool down to room temperature. Then, the seeds were kept in sealed bags and stored at 4-8°C in dark conditions until analysis. Seeds without roasting treatment were considered as control.

Fatty acid composition analysis

Fatty acid methyl esters were prepared from sesame seeds by following the method of Appelqvist (1968) and analyzed on Agilent technologies Gas Chromatography model 7820A series using CP-Sil 88 (25m x 0.25mm x 0.20mm) FAME column with a flame ionization detector in the presence of nitrogen as the carrier gas. The separation was carried out at 180-210°C (rate 4°C/ min). Temperatures of detector and injector were maintained at 240, and 230°C, respectively. The flow rate of nitrogen, hydrogen and air were 29ml/min, 30ml/min and 300ml/min, respectively. One microliters of sample was injected at a split ratio of 80:1. Individual fatty acids were expressed as percentages of the total fatty acids.

Total phenol content analysis

Total phenols were determined using Folin-Ciocalteu method by Swain and Hillis (1959). Homogenization of 0.5g sesame seed was carried out in 80% methanol (10ml) and refluxed on water bath for 2 h at temperature 70–75°C. The methanolic extract was pooled after refluxing, and total volume was made to 10ml by washing with 80% methanol. The methanolic extract (0.5ml) was mixed with 0.5ml of Folin-Ciocalteu reagent (2N) and saturated solution of Na₂CO₃ (1 ml), and centrifuged at 13,500 x g for 5min. The absorbance

of the upper phase was measured using a Techcomp UV- 2600 spectrophotometer at 760nm after 1h incubation at room temperature. The standard curve made with gallic acid (10-100µg) was used to calculate the concentration of the total phenol content (mg g⁻¹ gallic acid equivalent (GAE)).

Tocopherol analysis

Tocopherols were estimated as per method of Kayden *et al.* (1973). Seed sample (0.3g) were crushed in ethanol (3.0 ml), vortexed and centrifuged at 4000 rpm for 10min. Pipetted 1.2 ml of supernatant, standard Vitamin-E dl α-tocopherol standard solution (0.02mg/ml of absolute ethanol) and absolute alcohol for test, standard and blank, respectively. To each test tube, ddH₂O (1.2 ml) and purified xylene (1.2ml) was added and vortexed, followed by centrifugation at 5000rpm for 5min. The xylene layer (0.8 ml) was pipetted carefully into fresh tubes followed by addition of bathophenanthroline, FeCl₃, H₃PO₄ 0.4 ml each. The absorbance was read at 536nm against blank within 30sec. Purified xylene was used as reference blank. Avoid samples to come in contact with sunlight.

$$\text{Total tocopherol (}\mu\text{g/ g oil)} = \frac{A_T \times \text{standard concentration} \times \text{total volume of sample (3ml)} \times \text{percent}}{A_S \times \text{volume of test taken} \times \text{weight of tissue (g)} \times \text{oil content}}$$

A_T = Absorbance of test- absorbance of blank; A_S = Absorbance of standard- absorbance of blank

Total antioxidant capacity

The total antioxidant capacity of the methanolic extract prepared for phenols estimation was quantified using phosphomolybdenum method by Prieto *et al.* (1999). An aliquot of 0.1 ml of samples was mixed with 1 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and 1 ml of distilled water, and incubated in boiling water bath at 95°C for 90min. The reaction mixture was cooled to room temperature and the absorbance was read at 695 nm against blank using a Techcomp UV-2600 spectrophotometer. Total antioxidant activity in the sample was calculated by using standard curve of ascorbic acid(10-100 µg/ml) and expressed as mg g⁻¹.

DPPH radical scavenging activity

DPPH radical scavenging activity of the total phenol extracts was determined in accordance with Blois (1958). A mix of methanolic extract (0.1ml) and 0.1 mM DPPH solution in ethanol (5ml) was incubated for 20min at 27°C in dark. Absorbance of the sample and control (methanol and DPPH) was measured at 517 nm against methanol as a blank using a Techcomp UV- 2600 spectrophotometer. The inhibition percentage

was calculated using the following formula:

$$\% \text{ DPPH radical scavenging activity} = \frac{(1 - \text{sample absorbance})}{\text{control absorbance}} \times 100$$

Statistical analysis

The data of different parameters are expressed as means $\pm$ standard deviation of three replicates. The significant differences among means of unroasted (control) and roasted sesame seeds were analyzed using the GLM procedure and the Tukey-Kramer least means separation test (SAS software version 9.4).

RESULTS AND DISCUSSION

Nine cultivars of sesame seeds (*Sesamum indicum* L.) with variability in seed colour and oil content (Table 1) were used in the study. White colour sesame seeds (LT-16-296-1, LT-15-28, AT-338, RT-346, Pb Til No.2) has higher oil content ranging from 43.6 – 50.1% as compared to black and brown colour varieties (LT-16-132-1, LT-16-276-2, LT-16-374-1, LT-16-401-1) which showed variation in oil content from 35.4 to 38.7 % (Table 1). It has been well documented through previous reports (Zhang *et al.*, 2013; Kanu, 2011) that the light seeded samples are richer in oil than dark seeded ones. Sesame seeds are usually roasted as roasting improves the taste. It is believed that roasting at high temperature may lead to rancidification of oil present in the sesame seeds; however, roasted sesame seeds resist rancidity due to the antioxidants generated during seed roasting. Presence of these anti-oxidative compounds in sesame seeds imparts high oxidative stability. Also, roasting at very high temperature or for a long time may prove damaging to the nutritional value of the seeds. Our study was undertaken to optimize the roasting conditions for sesame seeds and the experimental responses are shown in Figs. 1 to 3.

Influence of roasting on fatty acid composition of sesame seeds

The stability and nutritional value of oil is significantly dependent upon its fatty acid profile. The predominant fatty acids in nine sesame samples under study were monounsaturated fatty acid (oleic acid, C_{18:1}) and polyunsaturated fatty acid (linoleic acid, C_{18:2}) varying from 38.3% (LT-16-276-2) to 44.2 % (AT-338) and 39.6% (AT-338) to 45.5% (LT-16-276-2), respectively. Oleic acid was slightly higher than linoleic acid in all the tested genotypes, except LT-16-276-2 and LT-16-374-1 which has high linoleic and less oleic acid. Saturated fatty acids (SFA) viz., palmitic (C_{16:0}) and stearic (C_{18:0}) was in the range of 14.9 to 16.8% with a lesser amount (<0.54%) of polyunsaturated fatty acid (linolenic acid, C_{18:3}). Application of roasting at 180, 200 and 220°C on oleic acid and linoleic acid that constitutes the major share of total fatty acids (>80%) is shown in Fig. 1. Roasting had no significant effect on fatty acid profile while maximum increase in the share of oleic acid was observed for roasting at 220°C for 30 min ranging from 0.9% (LT-16-401-1) to 5.1% (LT-16-132-1) with a corresponding decrease in linoleic acid from 1.3% (LT-16-401-1) to 5.2% (LT-15-28) compared to raw seeds. In agreement to our studies, Hama (2017) showed that roasting sesame seeds 200 °C for 30 min negatively affects the linoleic acid content with a corresponding increase in oleic acid. Alternatively, unchanged fatty acid compositions were reported in rice germ oil (Kim *et al.*, 2002), sesame seed oil (Yoshida and Takagi 1997, Mohamed and Awatif 1998) and safflower seed oil (Lee *et al.*, 2004) on roasting at different temperatures and time combinations. The ample amount of oleic and linoleic acid in sesame seeds is favourable in promoting of health benefits as both of these fatty acids produce hormones that reduces blood pressure and LDL cholesterol levels (O'Byrne *et al.*, 1997), hence the consumption of roasted sesame seed would serve as a

Table 1. Details of sesame varieties used in this study

S.no.	Genotype	Seed colour	Oil (%)	Place of collection
1.	PbTil no.2	White	49.4	PAU, Ludhiana
2.	RT-346	White	48.1	PAU, Ludhiana
3.	LT-16-132-1	Brown	35.4	PAU, Ludhiana
4.	LT-16-276-2	Black	36.1	PAU, Ludhiana
5.	AT-338	White	43.6	Amreli (Maharashtra)
6.	LT-16-401-1	Brown	37.6	PAU, Ludhiana
7.	LT-15-28	White	49.3	PAU, Ludhiana
8.	LT-16-296-1	White	50.1	PAU, Ludhiana
9.	LT-16-374-1	Black	38.7	PAU, Ludhiana

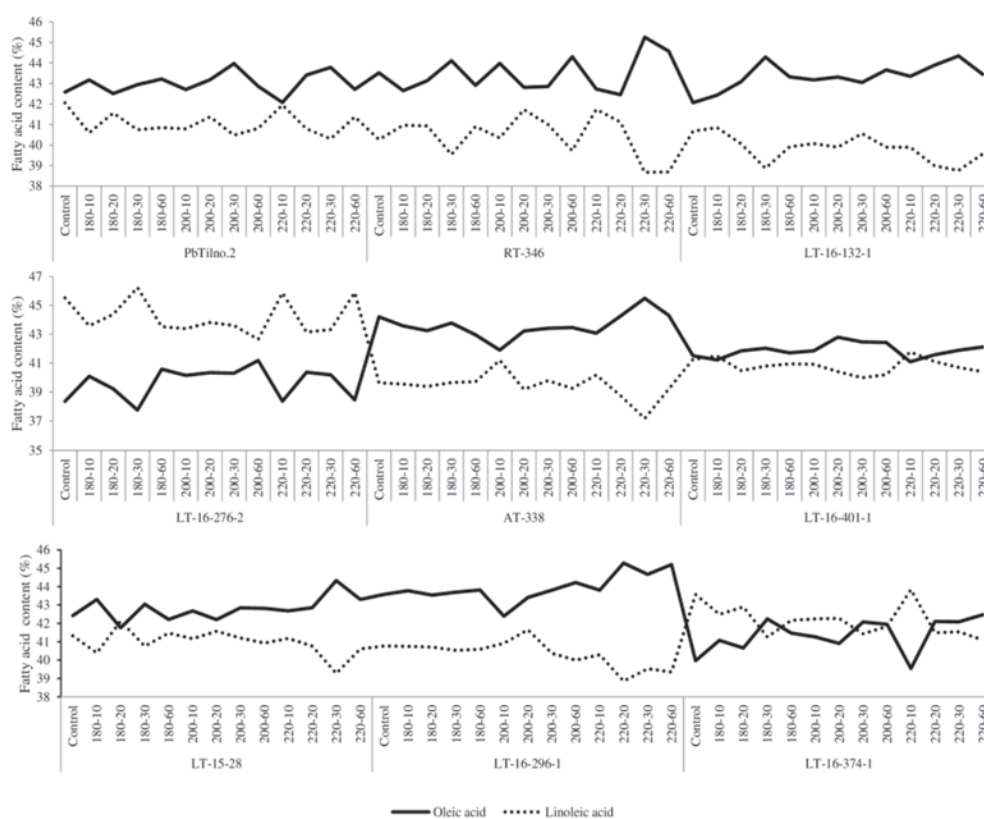


Fig. 1. Changes in monounsaturated (oleic acid, $C_{18:1}$) and polyunsaturated (linoleic acid, $C_{18:2}$) fatty acid composition of sesame seeds under different roasting conditions

good source of linoleic, an essential fatty acid.

Influence of roasting on total phenol content

The total phenol content in the methanolic extract of nine sesame cultivars was measured before and after roasting at 180 °C, 200 °C and 220 °C for 10, 20, 30 and 60 min ($n=117$). The increments were found to be higher in roasted seeds compared to unroasted ones indicating the effective release of antioxidative compounds. The amount of total phenol content varied in different sesame cultivars from 1.3 mg/g to 2.2 mg/g in RT-346 (white color) and LT-16-276-2 (dark color) cultivars, respectively. Genotypes namely, AT-338, LT-16-276-2, LT-16-401-1, LT-15-28 and LT-16-374-1 had more total phenol content than others. Highest percent increase was observed on roasting at 200°C for 60 min followed by 220 °C for 20 and 60 min (Fig. 2). A remarkable increase in total phenol content was clearly shown in roasted sesame seeds and was even more pronounced for sesame seeds roasted for a longer time. Increase in the total phenols on roasting was due to cleavage and liberation of phenols. A study by Hassan (2012) suggested formation of most desirable phenolic compounds on roasting sesame seeds in comparison to raw seeds. It was also noticed that effective processing step is required for liberating

antioxidative compounds as Lee *et al.* (2003) studies showed that far-infrared treatment to rice hull results in cleavage of tightly bound phenolics whereas simple heat treatment failed to cleave. Phenolic compounds are strong natural antioxidants as their presence prevents oxidative process, remove free radicals, and act as metal chelators (Teets and Were, 2008), thus prevents the oxidative damage and the formation of undesirable flavours and odors in the substrates (Brewer, 2011) and also consumption of such phenolic rich foods helps in protecting the body from the oxidative stress and therefore could play a significant role in human health.

Influence of roasting on tocopherol

Tocopherol is a potent antioxidative compound in vegetable oils, hence, the content of tocopherol in raw and roasted sesame seeds was necessary to be determined and the results are presented in Fig. 2. The tocopherol content ranged from 206 µg/g in LT-16-296-1 (white color) to 1125 µg/g in LT -16-374-1 (black) sesame cultivars, respectively. Though in all the nine sesame cultivars, availability of tocopherol content was enhanced significantly ($p < 0.05$) with the rise in roasting temperature and time. A high percent increase was observed on roasting at 220°C for 20 min and 60 min compared to unroasted seeds (control). Although

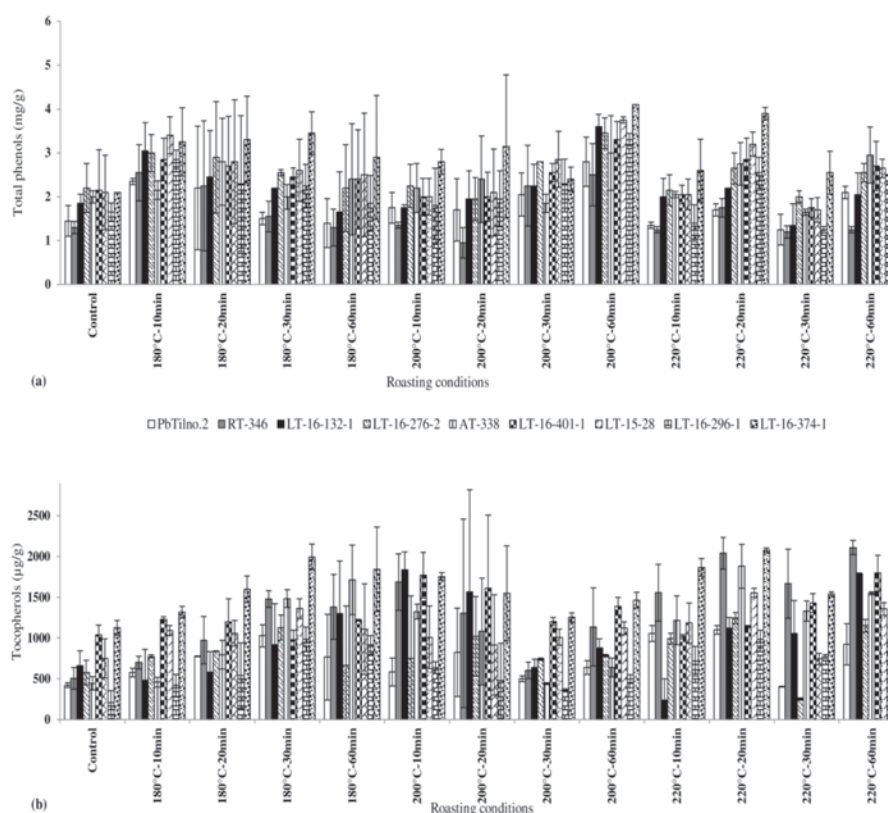


Fig. 2. Effect of roasting at different temperatures and periods on (a) total phenols (mg/g) and (b) tocopherols (µg/g) contents of sesame seeds

two cultivars; LT-16-401-1 and LT-16-374-1 have more tocopherol content than the other seven cultivars still the highest fold increase in tocopherol content was observed in LT-16-296-1 which genetically bears the lowest tocopherol content (205.75 µg/g) but showed 79.09% (984.05 µg/g) increase on roasting at 220 °C for 20 min and 78.94% (977.2 µg/g) at 220 °C for 60 min. The results show that the amount of tocopherol increased significantly as the roasting temperature and time; so the highest content will be achieved by roasting at 220 °C for 20 and 60 min. It was also observed that all the dark color genotypes (LT-16-132-1, LT-16-276-2, LT-16-401-1, LT-16-374-1) possesses more tocopherol content ranging from 575 to 1125 µg/g as compared to the light coloured ones (Pb Til No.2, RT-346, LT-16-296-1) from 205.75 to 747.6 µg/g, whereas a high fold increase of about 2.07-4.78 fold in tocopherol was revealed after roasting light coloured varieties compared to dark coloured ones (1.1-2.16) in comparison with control. The presence of such antioxidative compounds (tocopherols and lignan) in sesamum imparts remarkable oxidative stability to its oil (Konsoula and Liakopoulou-Kyriakides, 2010). Depending on the nature and variety of oilseeds, roasting may lead to a decrease (Simsek *et al.*, 2015) or an increase in oil tocopherols level (Kim *et al.*, 2002, Lee *et al.*, 2004). Due to the thermo-sensitive nature

of tocopherols, their final concentration depends upon the temperature and duration of roasting. Alternatively, better extraction procedures which disrupts the tocopherol retaining cellular structures and rupture their binding to membrane protein/ phospholipids could increase the tocopherols level (Belcadi-Haloui *et al.*, 2018).

Influence of roasting on total antioxidant activity

Roasting sesame seeds were found to have the greatest influence on the total antioxidant activity. The total antioxidant activity increased significantly ($p < 0.05$) with the roasting temperature. The range of activity varied in different sesame cultivars from 1.45 to 3.45mg/g in light coloured and 2.7 to 2.85 mg/g in dark coloured genotypes, respectively. Highest fold increase was observed on roasting at 220°C for 60 min (Fig. 3). The longer the roasting period of sesamum, the better is the antioxidant activity of the compound. After roasting for 60 min, the antioxidant activity of sesame seeds increased from 1.7 to 5.2 fold, compared to before roasting, contributing 43 % to 80.79% increase to the antioxidant activity. There have been studies showing that the antioxidant properties of plant extracts were enhanced during roasting. Lee *et al.*

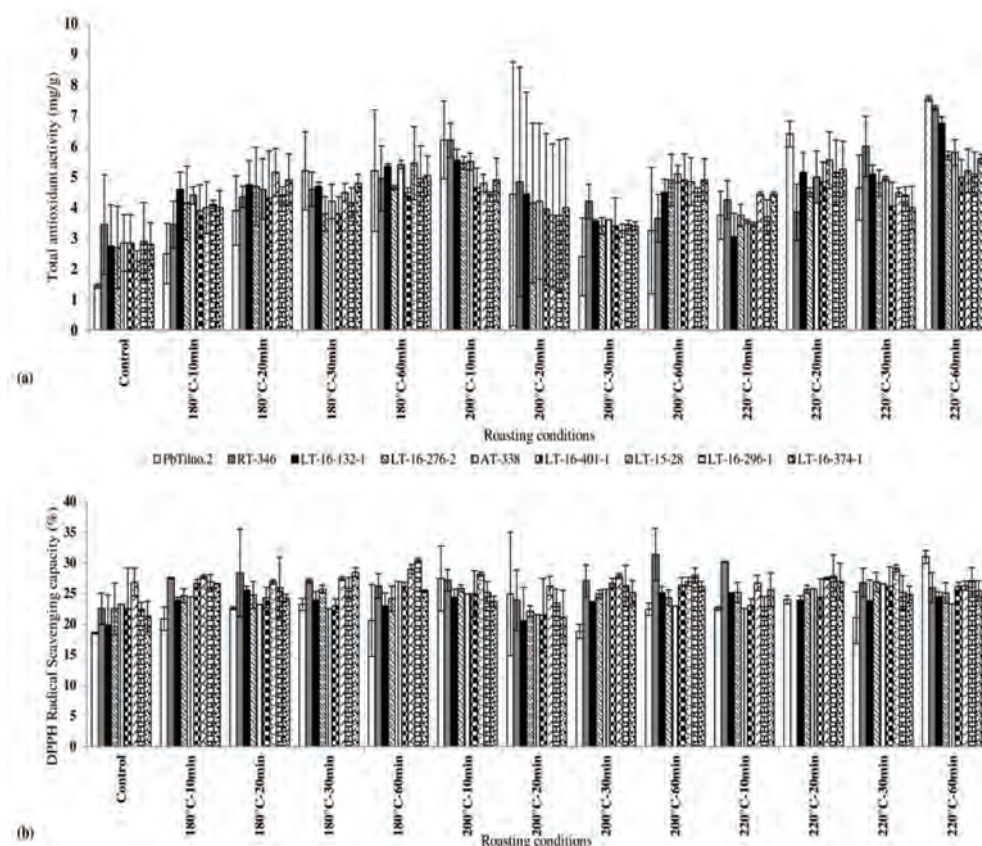


Fig. 3. Effect of roasting at different temperatures and periods on (a) total antioxidant activity (mg/g) and DPPH radical scavenging activity (%) of sesame seeds

(2013) found that roasting soybean at 200°C for 15min showed the highest antioxidant activity in comparison with other roasting conditions. Increased antioxidant property after roasting for 60 min might be because the breakdown of cellular constituents and membranes during heating allows more phenolics to be extracted. The Maillard reaction occurs during heating which allows the formation of a variety of intermediates and brown pigments (melanoidins), which might contribute to antioxidant properties (Chandrasekara and Shahidi, 2011).

Influence of roasting on DPPH radical scavenging capacity

The antioxidant activities of nine sesame cultivars have been evaluated using DPPH method. The range of DPPH values was between 18.5 (PbTil No.2) to 26.8 (LT-15-28) in sesame cultivars that differ significantly ($p < 0.05$). DPPH radical scavenging activities sesame seeds increased with increase in roasting temperature, but the increase was more pronounced when the seeds were roasted between 200°C for 10min and 220°C for 30 and 60 min (Fig. 3). The radical scavenging activities increased from 6.63% to 32.60% after roasting them at 200°C for 10 min, and from 7.90% to 16.59% and

0.21% to 40.23% after 200°C for 30 min and 60 min respectively. Roasting sesame seeds between 160°C and 200°C increases the oxidative stability of sesame seeds by raising its antioxidant potential. Hence, to obtain the highest antioxidant activity sesame seeds should be roasted between 200 to 220°C. Jeong *et al.* (2004) studies agreed with our results, as the DPPH radical scavenging activity of sesame significantly increased on roasting seeds at 200°C for 60min.

The present study recommends roasting of sesame seeds at 220 °C for 20 to 60 min to harness high nutraceutical benefits. The application of roasting may contribute to increased utilization of this crop for enhanced food and nutrition security in India and other developing countries. Incorporation and consumption of roasted sesame seed oils has a wide range of possible applications in the prevention of oil oxidation and extending the shelf life of oils or food products.

Authors' contribution

Conceptualization and designing of the research work (SS, SK); Execution of lab experiments (SS); Analysis of data and interpretation (SS, JD); Preparation of the manuscript (SS, JD, SK).

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