



Determination of Nutrient Content, *in vitro* Digestibilities, Anti-Nutrients, B-Carotene and Total Antioxidant Activity of Sesame (*Sesamum indicum*)

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Authors' contributions

This work was carried out in collaboration between both authors. Authors TB and AK designed the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Author TB managed the literature searches and analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Sesame (*Sesamum indicum*) is one of the valuable vegetarian sources of dietary protein with good quality amino acids that are very essential for growth. It possesses phytonutrients such as omega-6 fatty acids, flavonoids, antioxidants, and certain vitamins with potential anti-cancerous as well as health-promoting properties. Sesame seeds were analyzed for various nutritional parameters. The amount of moisture, protein, fat, ash, and fibre were found to be 4.17, 21.18, 46.64, 4.62, and 3.06 percent, respectively whereas calcium, iron, and zinc were 1294, 15.37, and 7.74 mg/100g, respectively. *In vitro* protein digestibility was estimated to be 79.50% whereas phytic acid was 247.37 mg/100g, and polyphenols 189.30 mg/100g. The values for β -Carotene and total antioxidant activity (TAA) was found to be 12.75 μ g/100g and 0.94 mg/g, respectively. Looking at the good nutritional profile of sesame, it can be utilized in various supplementary food products to enhance the product quality by improving its taste and increasing its energy, protein, calcium, and mineral content. Incorporation of sesame seeds into daily food items can make them both micro and macro nutrient-dense, which can be used for feeding people from all the age-groups. And also, the information provided may help plant breeders to develop improved varieties of sesame in the future.

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Keywords: Sesame seeds; nutritional composition; total minerals; protein digestibility; β -carotene; polyphenols; total antioxidant activity.

1. INTRODUCTION

Sesame (*Sesamum indicum*) is one of the valuable vegetarian sources of dietary protein with good quality amino acids that are very essential for growth. It is one of the oldest oilseed crops known to mankind. Sesame has numerous different species, most being wild and local to sub-Saharan Africa. The Food and Agricultural Organization (FAO) stated that the world production of sesame seeds was 6.1 million tonnes, in 2016, led by Tanzania, Myanmar, India, and Sudan [1]. This crop is liberal to dry conditions, developing where different crops fail [2,3]. With a rich, nutty flavor, it is a common ingredient in various cuisines across the world [4,5]. Along with high oil content, it has a good nutritional profile, with 21.7% protein, 43.05% fat, 4.13% ash, 16.99% dietary fibre, 12.94 μ g/100 g β -carotene, and 2174 Kcal [6]. Another name for sesame is gingelly seeds and is commonly known as 'Til' in India. They have been extensively employed in culinary as well as in traditional medicines for their nutritive, preventive, and curative properties.

Sesame is a primary source of phytonutrients such as omega-6 fatty acids, flavonoids, antioxidants, vitamins, and dietary fibre with potential anti-cancerous as well as health-promoting properties. It is noteworthy to express that sesame which is rich in poly unsaturated fats (PUFA), sesamin and vitamin E extraordinarily decreases hypertension when contrasted with the circulatory strain bringing down medications. It likewise diminishes histological renal harm and degeneration of the blood vessel, an element not seen in typical eating routine. Sesamin is significant for disease prevention and treatment of heart hypertrophy and renal hyper strain. Insufficient intake of nutritiously balanced food hinders growth [7,8].

Malnutrition and poor growth during early stages influence an expansive part of the world's total population. Prasad, et al. [9] Studied that nutraceuticals and pharmaceutical products of sesame can decrease the risk of neurological, dermatological, cancer, and heart disease. About 100 g of sesame seeds contribute a high amount of minerals, where calcium, iron, and zinc accounts for 1283, 15.05, and 7.7 mg, respectively [6]. Therefore, various supplementary food products can be prepared by

the addition of sesame to utilize their potential and enhance product quality by improving its taste and increasing its energy, protein, calcium, and mineral content. Incorporation of sesame seeds into daily food items can make them both micro and macro nutrient-dense, which can be used for feeding people from all the age-groups. Commercially available nutrient supplements are limited to certain circumstances but such foods can be consumed in daily routine. The objective of the study was to emphasize the nutritional profile of sesame to encourage its consumption of these traditional seeds. Therefore, the local cultivar of sesame was analyzed for its proximate composition, total minerals, *in vitro* protein digestibility, phytic acid, polyphenols, β -carotene, and total antioxidant activity.

2. MATERIALS AND METHODS

The present investigation was carried out in the Department of Foods and Nutrition, Chaudhary Charan Singh Haryana Agricultural University, Hisar. The sample for sesame was taken in a single lot from the local market.

2.1 Preparation of Sample

Sesame seeds were cleaned to remove foreign matter that could affect its nutrient analysis. The clean seeds were roasted in a pan on a medium flame for 4-5 minutes (until brown), the roasted seeds were allowed to cool at room temperature, followed by grinding them into a fine powder using an electrical mixer-grinder. To evaluate the nutrition profile of sesame, parameters analyzed included proximate composition (moisture, crude protein, crude fat, ash, and crude fibre); minerals (calcium, iron, and zinc); *in vitro* digestibility (protein and starch); phytic acid; polyphenols; β -carotene and Total antioxidant activity (TAA).

2.2 Nutritional Evaluation

2.2.1 Proximate composition

Analysis for moisture, crude protein, crude fat, ash, and crude fibre was done by employing the standard method of analysis [10].

2.3 Moisture

Ten g sample was weighed in a petri dish and dried in an oven at 105°C till a constant weight was obtained. The sample was weighed after cooling it in a desiccator.

$$\text{Moisture (\%)} = \frac{\text{Loss in weight}}{\text{Weight (g) of sample}} \times 100$$

2.4 Crude Protein

The crude protein was calculated by using the conversion factor of $N \times 6.25$.

Reagents: (i) Hydrochloric acid (N/100); (ii) Boric acid (4%); (iii) Sodium hydroxide (40%); (iv) Digestion mixture: 10g K_2SO_4 , 0.5g $CuSO_4 \cdot 6H_2O$, and 2g $FeSO_4$; (v) Mixed indicator solution: Dissolved 0.5 g of bromocresol green and 0.1 g of methyl red in 100 ml 95 percent ethanol and the solution was adjusted with drops of dilute NaOH to bluish-purple color.

Procedure: Two hundred mg sample was taken and digested with 20 ml concentrated H_2SO_4 and a pinch of digestion mixture. The nitrogen, as ammonical salt, was distilled with 40 percent NaOH in a Microkjeldahl apparatus. The ammonia thus liberated was absorbed in a 10 ml boric acid solution containing a few drops of mixed indicator and was titrated against standard HCl (N/100). The end-point was indicated by the change of color from bluish-green to pink.

$$\text{Crude protein (\%)} = \frac{0.00014 \times V \times (S-B) \times 100}{V_1 \times W} \times F$$

Where,

W = weight (g) of sample taken; V = volume (ml) made; V_1 = volume (ml) of aliquot taken for distillation; S = volume (ml) of HCl (N/100) used in titration for blank; B = volume (ml) of HCl (N/100) used in titration for blank; 0.00014 = 10ml of 0.1 N HCl neutralize 0.00014g of nitrogen; F = factor for converting N to protein (6.25).

2.5 Crude Fibre

Reagents: (i) Hydrochloric acid (%) v/v; (ii) Sulphuric acid stock solution (10%) v/v: Diluted 55 ml concentrated sulphuric acid to one litre; (iii) Sulphuric acid working solution (1.25%): Diluted 125 ml of stock solution to one litre; (iv) Sodium hydroxide stock solution (10%) w/v: Dissolved 100 g of NaOH in distilled water and diluted to one litre; (v) Sodium hydroxide working solution (1.25%): Diluted 125 ml stock solution to one litre with distilled water; (vi) Antifoam (2%): Silicon in CCl_4 .

Procedure: Two-gram fat-free dried sample was put in one litre tall beaker and to which 200 ml of 1.25 percent H_2SO_4 and a few drops of antifoam were added. The solution was kept for boiling for 30 minutes under bulb condenser. Beaker was rotated occasionally to mix the contents and remove the particles from the sides. The contents were filtered into the beaker through Buchner funnel. The sample was washed back into the beaker with 200 ml 1.25 percent NaOH and again boiled for exactly 30 minutes. All the insoluble mass was transferred to the sintered crucible (G-1) by means of boiling distilled water till acid-free. Washed twice with alcohol and thrice with acetone, and then dried at $100^\circ C$ to constant weight. The dried material was ashed in a muffle furnace at $550^\circ C$ for 1 hour. The crucible was cooled in a dessicator and weighed.

$$\text{Crude fibre (\%)} = \frac{W_2 - W_3}{W_1} \times 100$$

Where, W_1 = weight (g) of sample; W_2 = weight (g) of insoluble matter (wt. of crucible – insoluble matter – wt. of crucible); W_3 = weight (g) of ash (crucible + ash – wt. of crucible).

2.6 Crude Fat

Crude fat was estimated by using the soxhlet extraction apparatus.

Procedure: Five gram of moisture-free sample was taken and transferred to an extraction thimble and then weighed. The thimble was placed in a soxhlet extractor fitted with a condenser and flask containing sufficient petroleum ether. The extraction was carried out for six hours. After the extraction, the thimble was removed with the sample from the extraction apparatus and dried in a hot air oven to a constant weight. It was cooled in a dessicator and weighed. The loss in weight of the thimble was the estimate of the ether extract in the sample.

$$\text{Fat (\%)} = \frac{\text{Loss of weight (g)}}{\text{Sample weight (g)}} \times 100$$

2.7 Ash

Five gram of oven-dried sample was weighed in the silica crucible. It was ignited till no charred particles remained in the crucible. The crucible was put in a muffle furnace ($550^\circ C$) till a white ash was obtained (for 5-6 hours). The crucible was then cooled in a dessicator and weighed.

$$\text{Ash (\%)} = \frac{\text{Weight (g) of ash}}{\text{Weight (g) of sample}} \times 100$$

2.8 Total Minerals

Iron, zinc, and calcium were estimated using acid digested samples by Atomic Absorption Spectrophotometer AABQ-20 according to the method of [11]. Procedure: Two gram dried and ground sample was taken in a 150ml conical flask. To this, a 20ml diacid mixture ($\text{HNO}_3:\text{HClO}_4$: 5:1, v/v) was added and kept overnight. The next day it was digested by heating till clear white precipitates settled down at the bottom. The crystals were dissolved in double-distilled water. The contents were filtered through Whatman No. 42 filter paper. The filtrate was made to 50ml by adding double-distilled water.

$$\text{Minerals (mg/100g)} = \frac{\text{Reading (conc. } \mu\text{g/ml)} \times \text{volume made}}{\text{Weight of sample (g)} \times 1000} \times 100$$

2.9 In vitro Protein Digestibility

It was determined by the modified method of [12]. Reagents used: Pepsin reagent (0.1M KH_2PO_4 (pH 2.0) with 0.2 per cent pepsin); TCA (50%). Procedure: 250 of sample was weighed and transferred to a centrifuge tube. To it, 20ml of pepsin reagent was added. The tube was stoppered and arranged in a shaker incubator maintaining the water temperature at 37°C for three hours. Then the centrifuge tube was removed and cooled. 5ml of 50% TCA was added and centrifuged the contents at 10,000 rpm for 10 minutes at room temperature and filtered. Ten ml of aliquot was taken and dried in a hot air oven. Dried aliquot was digested for nitrogen determination by the Micro-kjeldahl method (10). The digested protein of the sample was determined. Protein digestibility was calculated employing the following formula:

$$\text{Protein digestibility (\%)} = \frac{\text{Digested protein}}{\text{Total protein}} \times 100$$

2.10 Phytic Acid

Phytic acid was determined by the method of [13]. Reagents: (i) Phytate reference solution: Exactly 30.54 mg sodium phytate (5.5% water, purity and containing 12 Na/mole) was dissolved in 100 ml of 0.2 N HCl which gave a solution containing 200 μg phytic acid per ml; (ii) Ferric

ammonium sulphate solution: Ferric ammonium sulphate (0.2g) was dissolved in 100 ml of 2N HCl and made the volume of 1000 ml with distilled water; (iii) Bipyridine solution: Ten gram 2-2 bipyridine and 10 ml thioglycollic acid were dissolved in distilled water and volume was made to 1000 ml. (These solutions are stable for several months at room temperature).

Extraction: Finely ground sample (0.5 g) was extracted with 25 ml of 0.2 N HCl for 3 hours continuous shaking in a shaker. Thereafter, it was filtered through Whatman # 1 filter paper.

Estimation: An aliquot (0.5 ml) of the above extract was pipetted into a test tube fitted with a ground glass stopper. One ml of ferric ammonium sulphate was added. The tube was heated in a boiling water bath for 30 minutes. The contents of the tube were mixed and centrifuged at 3,000 rpm for 30 minutes. One ml of supernatant was transferred to another test tube and 1.5 ml bipyridine solution was added. The absorbance was measured at 519 nm against distilled water. For plotting a standard curve, different concentrations (0.2 to 1.0 ml) of standard sodium phytate solution containing 40-200 μg phytic acid were taken and made to 1.4 ml with water O.D. of 0.342 corresponded to 80 μg phytic acid.

2.11 Polyphenols

Polyphenols were extracted by the method of [14]. Extraction: Defatted sample (500mg) was refluxed with 50 ml methanol containing one percent HCl for four hours. The extract was concentrated by evaporating on a hot water bath and brought its volume to 25 ml with methanolic-HCl. The amount of polyphenolic compounds was estimated as tannic acid equivalent according to Folin-Davis procedure [15]. Reagents: (i) Folin-Denis reagent: To 750 ml water, 100 g sodium tungstate, 20 g phosphomolybdic acid, and 50 ml phosphoric acid were added and heated and then refluxed for 2 hours. It was cooled and diluted to one litre; (ii) Tannic acid (stock solution): 100 mg of tannic acid was dissolved in water and made volume up to one litre. In order to have a working standard solution, a 20 ml stock solution was further diluted to 100 ml with water; (iii) Saturated aqueous sodium carbonate solution: Dissolved 350 g sodium carbonate in one litre hot distilled water at 70°C to 80°C, cooled and filtered through glass wool.

Procedure: The test solution (1.5 ml) was diluted with distilled water to 8.5 ml in a graduated test tube. After thorough mixing, added 0.5 ml Folin-Denis reagent and the tubes were well shaken. Exactly after 3 minutes, one ml of a saturated sodium carbonate solution was added and the tubes were thoroughly shaken again. After an hour, the absorbance was read at 725 nm on UV-VIS Spectrophotometer 118 using a suitable blank. If the solution was cloudy or precipitates appeared, it was centrifuged before readings were taken. A standard curve was plotted by taking 0.5 ml to 4.0 ml working tannic standard solution containing 10 µg to 80 µg tannic acid.

$$\text{Polyphenols (mg/100g)} = \frac{M \times V \times 100}{W \times V_1 \times 1000}$$

Where,

M = Concentration of extract elute obtained from graph; V = Volume of extract made (ml); W = Weight (g) of the sample; V₁ = Volume of extract aliquot taken (ml).

β-Carotene was estimated by the method of [10]. Reagent- Water saturated n-butanol –n-butanol and water was mixed in the ratio of 6:2 (v/v) and shaken vigorously. Then allowed to stand till it is separated into two phases; the upper clear layer is water saturated n-butanol. Procedure- (i) Ten grams of sample in was dispersed in 50 ml water-saturated n-butanol to make a homogenous suspension. (ii) It was shaken gently and allowed to stand overnight (16 h) at room temperature in dark. (iii) Suspension was shaken again and filtered through Whatman filter paper No. 1. (iv) The volume of the filtrate was made to 100 ml. (v) Absorbance (A) of the clear filtrate was measured at 440 nm in Spectronic-20/spectrophotometer using saturated n-butanol as a blank. (vi) Amount of β-carotene was calculated using the following equation: β-carotene content (ppm) = 0.0105 + 23.5366 × A.

Total Antioxidant Activity was estimated by the method of [16]. Reagents- 0.6 M sulphuric acid; 28 mM sodium phosphate mono basic anhydrous; 4 mM ammonium molybdate.

Standard curve for Estimation of Total Antioxidants: Ascorbic acid (1mg/ml) was used for making a standard curve for the estimation of Total Antioxidants.

- A defatted meal (0.1 g) was mixed with 2ml of 80% methanolic solution. After that, this mixture was homogenized properly and then centrifuged it at 3000rpm for 10 minutes. The supernatant was collected and the final volume was made up to 2ml. Now this methanolic extract will be used for all antioxidant assay.
- 500ul of methanolic extract is taken (concentration 500ug/ml)
- The final volume was made to 1 ml with 80% methanol.
- 2.5 ml of Phosphomolybdate reagent (0.6M Sulphuric acid, 28mM Sodium phosphate, 4mM ammonium molybdate (1:1:1) was added to the above.
- Test tubes were incubated in a water bath @95°C for 90 mins.
- The above tubes were cooled to RT, Abs was read at 695nm against a blank.

Total antioxidant capacity expressed as mg/g.

2.12 Data Analysis

The results were expressed in the form of the mean ± SD (Standard Deviation) [17]. The experiments were carried out in triplicates.

3. RESULTS AND DISCUSSION

Sesame seeds were analyzed for their nutritional composition. A high amount of protein and fat content was found i.e. 21.18 and 46.64 percent, whereas crude fibre, ash, and moisture were 3.06, 4.62, and 4.17 percent, respectively (Table 1). As per data obtained in the present study, moisture content was slightly less than the findings of an earlier study done by [18] who reported the moisture content of sesame to be 5.5%. The amount of protein was observed to be similar to the results given by [19] and higher than the results given by [18] who reported 16.1% protein. The results for crude fat and ash were quite similar to the values quoted by [20] and [13]. Whereas, results obtained by [21] were higher (26.63% fat, 23.32% protein, 7.37% moisture, 10.28% crude fibre). Adebowale, et al. [19] reported a wide range of values for moisture, ash, fat, protein, crude fibre, calcium, and iron for various varieties of sesame seeds and it ranged from 5-8.6%, 4-7 %, 11.5-20.5%, 13.3-23%, 1.9-2.5%, 5.4-20.2 mg/kg, and 1.1-3 mg/kg, respectively.

Table 1. Nutrient content of sesame (mg/100 g, on dry matter basis)

Nutrient	Amount
Moisture (g/100g)	4.17±0.27
Crude Protein (g/100g)	21.18±1.41
Crude Fat (g/100g)	46.64±1.38
Ash (g/100g)	4.62±0.27
Crude Fibre (g/100g)	3.06±0.38
Calcium (mg/100 g)	1294±5.37
Iron (mg/100 g)	15.37±.34
Zinc (mg/100 g)	7.74±.33

Values are means ± SE of three observations

Results of the present investigation were in support of sesame to be considered as a good mineral contributor with 1294mg/100 g calcium, 15.37 mg/100g iron, and 7.74 mg/100g zinc content. The results for minerals were almost similar to the values reported by [6] and slightly higher than the amount quoted by [18]. Alyemeni, et al. [20] estimated calcium, iron, and zinc to be 1200, 10.6 mg/100 g, and 3.8 mg/100g in sesame seeds.

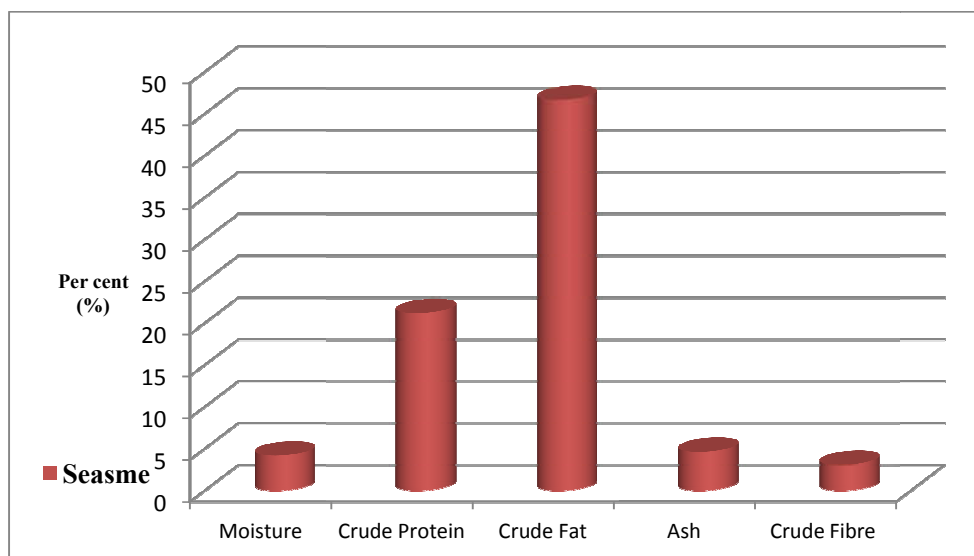
The nutritional quality of sesame becomes limited due to the existence of anti-nutritional factors that exhibit adverse physiological effects [22,7]. Anti-nutrients like tannins and phytic acid, influence mineral utilization [23,24]. The negatively charged phytate at the physiological pH binds the positively charged ions of calcium, iron, magnesium, and zinc that are nutritionally essential and important. This leads to the

formation of insoluble complexes, which further leads to the unavailability of minerals for absorption [25]. Therefore, removal or reduction of such unwanted components from food is necessary. Roasting is one of the thermal processes that could be easily employed even at domestic use to serve the motive. As per data (Table 2), phytic acid was observed to be 247.37 mg/100g. Roasting is considered to be an effective method for reducing the phytic acid content in the sesame and plant-based foodstuffs [25,26,27,28]. In addition to roasting, other thermal processings effective for reducing anti-nutrients and improving *in-vitro* digestibility include pressure-cooking, microwave, auto-claving, and boiling [29]. He found phytic acid widely ranging from 1.98-6.5 g/100g in differently processed and unprocessed sesame seeds. Previously, [30] and [31] reported phytic acid in raw sesame seeds to be 4.7 and 5.36 g/100g, respectively.

Table 2. Nutrient content of Sesame (on dry matter basis)

Nutrient	Sesame
Protein Digestibility (%)	79.50±1.78
Phytic acid (mg/100 g)	247.37±0.93
Polyphenols (mg/100 g)	189.30±.65
β-Carotene (µg/100g)	12.75±0.85
Total Antioxidant Activity (mg/g)	0.94±0.02

Values are mean ± SE of three independent determinations

**Fig. 1. Proximate composition of sesame (g/100 g, on dry matter basis)**

Values are mean ± SE of three independent determinations

Protein digestibility is crucial in evaluating the nutritive quality of a food protein, as it is a chief determinant of the availability of amino acids. *In vitro* protein digestibility (IVPD) for sesame seeds was found to be 79.50% (Table 2). Our value for IVPD was lower than observed by [29]. He noticed, roasting partially eliminated and reduced different anti-nutritional factors after processing sesame, and improved IVPD to 85%.

The amount of polyphenols in the methanol extracted sample of sesame was recorded to be 189.30 mg/100g (Table 2). Zhou, et al. [32] Reported total phenolic contents in black sesame varieties ranged from 45.4 to 73.2mg/GAE/100g. Total phenols of sesame in the methanolic extract were reported to be 19.48 mg/g by [33]. Khan, et al. [34] Analyzed the total phenolic content (TPC) in methanol extract to be varying between 68.06-168.40 mg GAE/100g for various genotypes of sesame seeds. Samuel and Genevieve [21] Found 13.83mg/100g phenols. [35] Determined TPC in six varieties of white sesame to be 370.5-786.8 mg GAE/ 100g. Earlier, TPC of black and white sesame seed cultivars were reported to be 299 and 106 catechin equivalents/100g crude ethanolic extract by [36]. The findings for β -Carotene i.e., 12.75 μ g/100g (Table) was in proximity to the results given by [6] (12.94 μ g/100g).

The total antioxidant activity (TAA) of sesame in methanol extraction was found to be 0.94, mg/g in the present study (Table 2). Varied results were recorded by other researchers based on different procedures and standards used for the analysis of TAA. DPPH radical scavenging activity of sesame was between 4.01-25.50 mg/ml for various varieties of sesame seeds as given by [34]. [37] Analyzed 0.026 mg/ml DPPH radical scavenging activity in sesame oil extract.

The nutritional importance and health benefits of sesame seeds are being explored for a very long time and still ongoing. A lot of researchers [18,38,39,40] have utilized it in making value-added food products. [38] Developed traditional snack incorporating sesame for nutrient enrichment; [39] used sesame for the fortification of weaning food; [18] developed various kinds of traditional products and instant mixes by adding sesame in varied content; [40] supplemented various value-added products like *laddoo*, *burfi*, *chapati*, *halwa* with sesame seeds. All the researchers obtained acceptable sensory results with a good nutritional profile of the developed value added food products. Thus, the diverse

use of sesame is possible in various kinds of recipes. Reviewing the available literature uncovered that there is a lack of literature on the physico-chemical and mineral composition of this significant oilseed crop which might affect its current utility. Therefore, highlighting the nutritional importance of sesame may encourage its consumption and acceptance in daily routine recipes.

4. CONCLUSION

The current study reveals that sesame contains a good amount of micro and macro nutrients. These seeds are rich in energy, protein, calcium, iron, zinc, and adequately possess polyphenols, β -carotene, and free-radical scavenging activity. Subjecting the seeds to thermal treatments like roasting, boiling, microwave, pressure cooking, and autoclaving reduces the amount of anti-nutrients that results in the improvement of protein digestibility. The information provided may help plant breeders to develop improved varieties of sesame in the future. The evaluated nutritional parameters of the sesame seed have favorable results that can authenticate its recommendation for usage in a wide range of foodstuffs. Incorporation of sesame seeds into daily food items can improve their nutrient-density, which can be used for feeding people from all the age-groups. Consumption and utilization of sesame could prove to be beneficial in combating the global problems like protein energy malnutrition and micro-nutrient deficiencies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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