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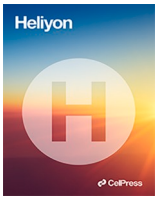

Research article

# Antioxidant activity, physico-chemical properties, and bioactive compounds of *Nigella sativa* seeds and oil impacted by microwave processing technique

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A R T I C L E I N F O

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A B S T R A C T

Strongly anti-oxidant and medicinal, *Nigella sativa* L (NS) is utilized in conventional medicine to address a range of illnesses, including gastrointestinal, inflammatory and rheumatic illnesses. This study was carried out to investigate the effects of microwave processing on the physico-chemical properties of Moroccan-grown Nigella sativa seeds and oils, as well as to investigate the anti-oxidant qualities of black cumin oils under conditions of accelerated oxidation. The study's specific goal was to ascertain the effects of varying microwave power levels (500 and 750 W) and roasting times (5, 10, and 15 min) on the black cumin oils' quality indices, fatty acid and sterol content, carotenoid and chlorophyll levels, mineral profile, tocopherol amount, and overall antioxidant activity. To this end, the seeds of black cumin were roasted at two power levels (500 and 750 W) and for three different periods (5, 10, and 15 min) in a microwave oven. The obtained results show that the duration and the processing power did not significantly influence the amount of sterols and fatty acids. In contrast, the quality indices, physico-chemical properties, carotenoid and chlorophyll contents, mineral profile, and tocopherol amount were influenced by the microwave processing. A significant decline in the antioxidant activity was recorded from 45.01 ± 0.81 % (unroasted cumin seeds) to 4.32 ± 0.91 % (750 W/5 min). Based on these findings, the black cumin oil preparations should be handled carefully and the oil must be protected once extracted. The stability and preservation of antioxidants are crucial steps against pro-

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oxidant and inflammatory conditions that could favour cellular senescence and accelerate aging processes.

## 1. Introduction

The seed of black cumin called black seed (*Nigella sativa* L.), is a famous herbaceous plant in the Ranunculaceae family, renowned or a long time for ethnomedicinal applications [1]. It is cultivated in many Middle Eastern and Mediterranean countries like Iran, India, Syria, Pakistan, and Saudi Arabia, [2]. Growing consumer preference for natural and herbal remedies contributes to the market's success [3]. In 2021, the global black seed oil market was estimated at 18.0 million USD, and from 2022 to 2028, it is expected to expand at a compound annual growth rate (CAGR) of 7.2 % [4]. *N. sativa* is certified for food use by the U.S. Food and Drug Administration (FDA), and has also been deemed generally recognized as safe (GRAS) by the Flavor and Extract Manufacturers Association (FEMA). *Nigella sativa* (NS) has a unique nutrient profile with antioxidant phytochemicals, and it can be used in food preparations to increase consumer health by improving the stability of food formulations and the levels of key nutrients [5,6]. The seeds are very oil-rich, which has many favorable effects on the human health due to its major (essential fatty acids) and minor (phenolic compounds, sterols, and tocopherols) compounds. Chemical composition of NS seed is well documented, several published works have reported the contents of Ash, moisture, protein, oil, and total carbohydrate in the following ranges 3.7–4.86, 3.8–8.65, 20.8–26.7, 24.48–40.35, and 24.9–40.0 g/100g, respectively [7,8]. The seeds also contain steryl glucosides, steryl esters, free sterols, while acylated steryl glucosides were found from the seed oil [9].

In the Arabian Peninsula, Far East Asia, Europe, and Africa, NS seed has long been utilized as a traditional remedy [10]. *Nigella sativa* was known as "The herb from heaven" and was considered a miraculous plant by early herbalists [11]. The black seed possesses healing properties as the Prophet Mohammed (PBUH) stated: "Hold on to use this black seed, as it has a remedy for every illness except death." [11].

So far NS has been extensively studied to prove its traditional claims [12]. It is used for many medicinal treatments, such as inflammatory disorders, gastrointestinal and rheumatic, as well as cardiovascular diseases, worm infections, cancer, anorexia, bronchitis, asthma, chronic headache, coryza, colic, coughs, dermatosis, dispiritedness, fever, diarrhea, eye infections, fatigue, and diabetes [13]. Many in vivo studies suggested that the black cumin seed oil has antioxidant, anti-inflammatory, antihypertensive, antidiabetic, antimicrobial, anticancer, diuretic, and analgesic activities [14]. In Pakistan, Morocco, and Southern Europe, NS is also used to treat many kinds of respiratory disorders [15]. Recently, NS has been used in the treatment of COVID-19, and some studies have reported that thymoquinone, nigellidine, and α-hederin from *N. sativa* may have a molecular role in enhancing the immunological response [16].

NS has oregano-like quality with slight bitterness and a warm, toasted-onion flavor. It frequently appears in food components for cheese, bakery products, yogurt, pastry, pickles, marinades, sauces, and salads [13]. The process of oil extraction from microwave heated seeds is highly significant as demonstrated by recent studies on pretreatment of chia seeds [17], chickpeas [18], sunflower seeds [19] and pomegranate seeds [20]. In recent years, heat treated or roasted products before oil extraction were generally conducted in order to inactivate enzymes, coagulate protein, impart aroma and flavor to the oil, facilitate the release of constituents of oil during the extraction process, or to increase the oil yield [21]. Numerous investigations looked into the influence of traditional oven roasting on physico-chemical properties and nutritional composition of nigella seed oil [22]. However, few works have focused on the impact of microwave roasting on nigella seed oil [22], this is particularly important because this treatment is increasingly used. Consequently, understanding of how microwave processing affects the oil's antioxidant properties and overall quality is important to protect the health of the consumers. In this optic this study was conducted and aims to investigate the impact of microwave processing (500 and 750 W) and roasting duration (5, 10, and 15 min) on antioxidant activity, physico-chemical properties, proximate composition, mineral and lipid profiling of black cumin seeds and oil of *Nigella sativa* plants cultivated in Morocco. The optimization of the oil processing technique conditions is a key step in studying and determining the stability of antioxidant compounds, and the quality of oils during heat treatments.

## 2. Material and methods

### 2.1. Plant material and sampling

Seeds were collected in the agricultural province of Souk Sebt Ouled Nemma (region of Beni Mellal), North-Central of Morocco (32°20′14″N, 6°20′59″W), in June 2019. After harvest, the cumin seeds were separated by all foreign materials (e.g., leaves, small stones), and stored in airtight bags at 4 °C.

### 2.2. Sample preparation

At a controlled room temperature (25 °C), 500 g of previously cleaned black cumin seeds were placed in a porcelain crucible. The seeds were exposed to microwaves set at two power levels, 500 and 750 W, for three exposure times, i.e. 5, 10 and 15 min. The microwaves were calibrated to ensure constant power levels. To ensure reproducibility, each experiment was repeated twice by the same experimenter, on the same day, with detailed documentation of the experimental conditions and procedures followed. After

roasting, the seeds were cooled to 25 °C, stored in plastic bags, and refrigerated at 4 °C until further use. The extraction of nigella seed oil was performed by mechanical press (Komet DD 85 G press, IBG Monforts Oekotec GmbH, Mönchengladbach, Germany). The extracted oil was used for the roasting effect evaluation of the oil composition and subsequently to determine the appropriate roasting conditions (power/time). After extraction, the nigella seed oil samples were kept in dark glass bottles of 30 mL.

### 2.3. Analytical methods

#### 2.3.1. Oil, moisture, protein and ash content

A quantity of 20 g nigella seeds were used for extraction using a Soxhlet apparatus and n-hexane as a solvent for 8 h [23]. Afterwards, the rotary evaporator (Büchi, R-100, Switzerland) was used to remove the solvent. The extracted oil was purged with nitrogen and kept in storage at −4 °C until further analysis. The extraction yield (oil content) was determined using formula (1):

$$\text{Oil content (\%)} = \text{weight of extracted oil/weight of sample x 100} \quad (1)$$

Moisture content was determined according to the official analytical method [24]. In brief, Binder's laboratory dryer was used to heat up 5 g of powder samples, and the results were determined using the equation below (2):

$$\text{Moisture content (\%)} = (m_2 - m_3) / (m_2 - m_1) \text{ x } 100 \quad (2)$$

Where $m_1$ is the mass of the empty crucible (g); $m_2$ is the mass of the crucible with sample before drying (g); $m_3$ is the mass of the crucible with sample after drying (g).

Protein content (PC) was determined using an analyzer LECO FP628 (LECO Corp., MI, USA) based on Dumas approach [25]. Nitrogen was converted to PC and expressed as a percentage of sample dry weight using a factor of 6.25.

Ash content determination was carried out using a muffle furnace by burning 1 g of the sample at 500 °C for 4 h [26].

#### 2.3.2. Carbohydrates content (CC) and energy value (EV)

According to a previous approach [27] the CC of the samples was estimated by subtracting the total of the other elements (oil, moisture, proteins, and ash), using the formula below (3):

$$\text{CC (\%)} = 100\% - (\text{Oil} + \text{Moisture} + \text{Proteins} + \text{Ash}) \quad (3)$$

EV (expressed in kcal/100 g of dry matter) was determined employing a method described earlier [28], using the following equation (4):

$$\text{EV} = (2.62 \text{ x PC}) + (8.37 \text{ x oil}) + (4.2 \text{ x CC}) \quad (4)$$

#### 2.3.3. Mineral profiling

The study of Mineral Profiling was performed using a PerkinElmer Model Optima 8000 DV spectrometer. First, 1g of sample powder was burned for 4 h at 525 °C in a muffle furnace. The resulting ash was then treated with 10 mL of HCl and 4 mL 65 % NHO3.

#### 2.3.4. Oil physico-chemical indices

The quality indices, including peroxide value (PV), acidity value (AV), UV absorption (K232) and para-anisidine value (p-AV), were analyzed according to the analytic methods described in the International Standard Organization [29–33].

AV was expressed as 1 g of oleic acid per 100 g of oil (g/100 g oil). PV was expressed as milliequivalents of active oxygen per kilogram of oil (mEq $O_2$/kg oil), and UV absorption K232 was measured in a 1 cm cuvette using a SCILOGEX SP-UV1100 spectrometer as the specific extinction of an oil in cyclohexane solution at 1 % (w/v) (Scilogex, CT, USA).

The evaluation of oil's secondary oxidation products is known as p-AV. P-anisidine standard in glacial acetic acid was left to react with an oil solution in iso-octane to produce yellowish reaction products, which were then used to detect p-AV. Then, using a SCILOGEX SP-UV1100 spectrometer, the absorbance observed at 350 nm in a 1 cm cuvette was used to investigate p-AV.

#### 2.3.5. Fatty acids, sterols, and tocopherols

Fatty acid content was determined following official analytical method [34]. Fatty acids were converted into fatty acid methyl esters and the composition was determined as their corresponding methyl esters by gas chromatography (Agilent 6890) on a CPWAX 52CB column (30 m × 0.25 mm i.d., 0.25 μm film thickness) using helium (He) as a carrier gas (flow rate 1 mL/min) and flame ionization detector (FID). 1 μL of sample was injected at 220 °C with split ratio of 1/50. Oven temperature was set at 180 °C for 5 min and was increased to 220 °C with a 15 °C/min slope. Detection was realized at 230 °C. Results are expressed as the relative percentage of the area of each individual fatty acid peak.

Sterol content was determined using the ISO method [35]. 50 mL of an ethanol potassium hydroxide solution (2 N) was used to saponify 5 g of oil by heating it under reflux for 1 h 100 mL of water was then added, and 200 mL of hex-ane was used to extract the unsaponifiable material. The organic solution was then collected, evaporated, and 20 mg of unsaponifiable were dissolved in 0.5 mL of chloroform. After that, the mixture was chromatographed onto a silica gel plate and eluted with a 65:35 v/v mixture of n-hexane and diethyl ether. The plate was then sprayed with a solution of 2,7- dichlorofluorescein (0.2 % in ethanol), and the sterol band was

carefully removed. The silica gel recovered from the plate was suspended in chloroform and filtered. The solvent was evaporated under N2, after trimethylsilylation the sterol composition was determined using a Varian 3800 instrument equipped with a VF-1 ms column (30 m × 0.25 mm inner diameter, 0.25 μm film thickness) and using helium as transport gas (flow rate 1.6 mL/min). The column temperature (270 °C) was isothermal, the injector and detector temperature were 300 °C. The amount injected was 1 μL in a split mode (split ratio 1:50). Retention time was used for identification, and internal standards (α-cholestanol) were used to quantify the total sterol. Results were given in mg/100 g of oil [36].

Tocopherol content was determined with HPLC (Shimadzu instruments fitted with a C18-Varian column (25 cm × 4 mm)). A fluorescence detector (excitation wave length: 290 nm, detection wave length: 330 nm) was used for the detection process. At a flow rate of 1.2 mL/min, an isooctane/isopropanol (99:1, v/v) mixture served as the eluent [37]. Retention time was used for identification, while external standards were used for tocopherol measurement. The results were given in mg/100 g.

#### 2.3.6. Chlorophyll and carotenoid contents

Chlorophylls and carotenoids in unroasted and microwave-roasted cumin seed oil were estimated from the absorption maxima of the extracted pigments in cyclohexane as described by Borello and Domenici [38]. Chlorophyll and carotenoid contents were measured using a spectrophotometer at wavelengths of 670 and 470 nm, respectively. The results were expressed as mg/100g oil.

#### 2.3.7. Total phenolic content and antioxidant activity

First, 1 g of defatted sample was mixed with 4 mL of methanol/water (60:40, v/v). A 125 μL intake of the methanol extract was mixed with 500 μL of distilled water and 125 μL of the reagent of Folin-Ciocalteu. After vigorous stirring of the mixture followed by standing for 3 min, an intake of 1250 μL of 7 % $Na_2CO_3$ was added. The solution was placed at room temperature for 90 min. Absorbance was recorded at 760 nm using a spectrophotometer. The total phenolic content of the samples was expressed as gallic acid equivalents (mg GAE/g) [39].

Antioxidant activity of samples was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition. Briefly, a sample of 50 μL and 50 μL of DPPH dissolved in methanol solution were added to make up to one using double distilled water. After incubation in the dark, at room temperature for 30 min, the absorbance (Abs) was recorded at 515 nm and the antioxidant activity (AA) was determined using equation (5):

$$AA\ (\%) = (Abs_{control} - Abs_{sample})/Abs_{control} \times 100 \quad (5)$$

### 2.4. Statistical analysis

Results are the mean values ± SD (standard deviation) of 3 replicates. The significance level was set at $p = 0.05$. Using Minitab software (version 17), the Duncan test was used to separate mean values at the 0.05 level of significance, histograms are plotted using originPro 2024 software.

## 3. Results and discussions

### 3.1. Mineral composition

The mineral content of unroasted and roasted black cumin seeds are presented in Table 1. Potassium (K) is the major constituent of the mineral elements of the cumin seeds with a content of 849.64 ± 17 mg/100 g for the unroasted seeds. This value is almost the same at 500 W during the three exposure times, while at 750 W the potassium content recorded a remarkable evolution which reached 2261.01 ± 53 mg/100 g after 15 min.

Calcium (Ca) is another mineral found in cumin seeds, with a value of 523.01 ± 4 mg/100 g for unroasted seeds. In roasted seeds at

**Table 1**
Effect of microwave roasting on mineral elements in black cumin seeds (mg/100g).

| UNS | | 500 W | | | 750 W | | |
|---|---|---|---|---|---|---|---|
| | | 5 min | 10 min | 15 min | 5 min | 10 min | 15 min |
| **Ca** | $523.01 \pm 4^{g}$ | $527.26 \pm 4^{f}$ | $547.96 \pm 2^{e}$ | $564.02 \pm 6^{d}$ | $652.34 \pm 7^{c}$ | $799.1 \pm 7^{b}$ | $1471.46 \pm 7^{a}$ |
| **Na** | $11.04 \pm 1^{g}$ | $13.57 \pm 4.5^{d}$ | $12.33 \pm 1.3^{e}$ | $11.66 \pm 9.8^{f}$ | $15.43 \pm 1.2^{c}$ | $24.1 \pm 4.5^{b}$ | $39.0 \pm 0.8^{a}$ |
| **K** | $849.64 \pm 17^{g}$ | $893.19 \pm 1^{f}$ | $926.37 \pm 12^{e}$ | $961.75 \pm 19^{d}$ | $1009.69 \pm 10^{c}$ | $1242.48 \pm 12^{b}$ | $2261.01 \pm 53^{a}$ |
| **Mg** | $258.9 \pm 1^{g}$ | $260.25 \pm 2^{f}$ | $271.63 \pm 25^{e}$ | $285.29 \pm 5^{d}$ | $311.8 \pm 14^{c}$ | $385.41 \pm 12^{b}$ | $698.41 \pm 9^{a}$ |
| **Fe** | $9.31 \pm 1^{d}$ | $8.87 \pm 0.1^{d}$ | $6.29 \pm 1^{f}$ | $7.0 \pm 0.2^{e}$ | $32.94 \pm 3.2^{a}$ | $19.52 \pm 5.4^{c}$ | $22.96 \pm 0.1^{b}$ |
| **Mn** | $19.77 \pm 0.1^{c}$ | $1.99 \pm 1.2^{c}$ | $2.03 \pm 0.17^{c}$ | $2.11 \pm 0.1^{c}$ | $2.76 \pm 0.1^{b}$ | $3.11 \pm 0.7^{b}$ | $5.58 \pm 0.1^{a}$ |
| **Cu** | $1.34 \pm 0.1^{c}$ | $1.42 \pm 0.4^{c}$ | $1.43 \pm 0.7^{c}$ | $1.46 \pm 0.9^{c}$ | $1.58 \pm 0.8^{c}$ | $2.14 \pm 0.2^{b}$ | $3.94 \pm 1.0^{a}$ |
| **Zn** | $6.03 \pm 0.1^{d}$ | $6.14 \pm 1.3^{d}$ | $6.34 \pm 2^{d}$ | $6.66 \pm 0.2^{cd}$ | $7.18 \pm 12^{c}$ | $9.05 \pm 1.5^{b}$ | $16.53 \pm 1.0^{a}$ |
| **B** | $2.91 \pm 1.1^{d}$ | $3.02 \pm 1.5^{cd}$ | $3.11 \pm 1{,}2^{cd}$ | $3.18 \pm 1.1^{cd}$ | $3.51 \pm 1.2^{c}$ | $4.91 \pm 1.7^{b}$ | $7.93 \pm 1.6^{a}$ |
| **P** | $482.3 \pm 2^{g}$ | $490.2 \pm 13^{f}$ | $509.68 \pm 30^{e}$ | $534.94 \pm 37^{d}$ | $551.49 \pm 12^{c}$ | $685.23 \pm 10^{b}$ | $1106.53 \pm 60^{a}$ |

UNS: Unroasted Nigella Seeds; data values with the same alphabetic exponent within a row do not vary significantly ($P < 0.05$).

500 W for 5min, the calcium content did not change significantly and slightly increased to 564.0.2 ± 6 mg/100 g at 15 min. However, at 750 W the calcium content increased to 1471.46 ± 7 mg/100 g after 15min of roasting (Table 1). Phosphorus (P) is essential for plant cells and was found in mineral or organic form [40]. According to our results, the levels of phosphorus also increase with microwave roasting. At the beginning a value of 482.36 ± 2 mg/100 g was found in the unroasted seeds, but the phosphorus content goes up to 534.94 ± 37 mg/100 g and 1106.53 ± 60 mg/100 g for 500W and 750 W and 15 min of roasting, respectively.

Magnesium (Mg) was present in the seeds of black cumin at levels ranging from 258.9 ± 1 mg/100 g for the unroasted seeds, to a maximum value of 698.41 ± 9 mg/100 g of roasting at 750 W, after 15 min. The iron (Fe) content in unroasted seeds was 9.31 ± 1.0 mg/100 g, but decreased to 8.87 ± 0.1 mg/100 g and 7.0 ± 0.2 mg/100 g at 500 W for 5min and 15 min, respectively, while at 750W for 15 min, the iron content was 22.96 ± 0.1 mg/100 g. The zinc (Zn) content of the unroasted nigella seeds was 6.03 ± 0.1 mg/100 g. After roasting for 15 min, it became 6.66 ± 0.2 mg/100 g and 16.53 ± 1.0 mg/100 g in a power of 500 W and 750 W, respectively. The same observation could be noticed for Mn, Cu, and B in black cumin seeds. Considerable increased quantities were observed at 750 W/15 min.

These outcomes revealed that the microwave processing affected the mineral levels. The content is almost stable at 500 W during different time periods of roasting. However, at 750 W, a considerable change in the levels of mineral elements was noted as a function of roasting time. For all mineral elements in black cumin seeds, the content increased after the microwave treatment compared to the unroasted seeds. The increase in mineral content in black cumin seeds after microwave roasting may be due to several factors. Microwaves cause rapid evaporation of moisture, concentrating the minerals in the seeds [41]. In addition, the rapid, uniform heating alters the cell structure, facilitating the release and accessibility of minerals. This treatment can also induce specific chemical transformations that increase the bioavailability of mineral elements [42]. Compared with traditional roasting, this method could therefore better preserve or concentrate the minerals present. These outcomes are in agreement with previous investigations that found mineral content increase in different plant matrices after the roasting treatment [36,37,43]. Additionally, the iron content also increased after roasting in cocoa beans [44]. The differences between these studies could be attributed to variations in the seed types and compositions, as well as roasting conditions.

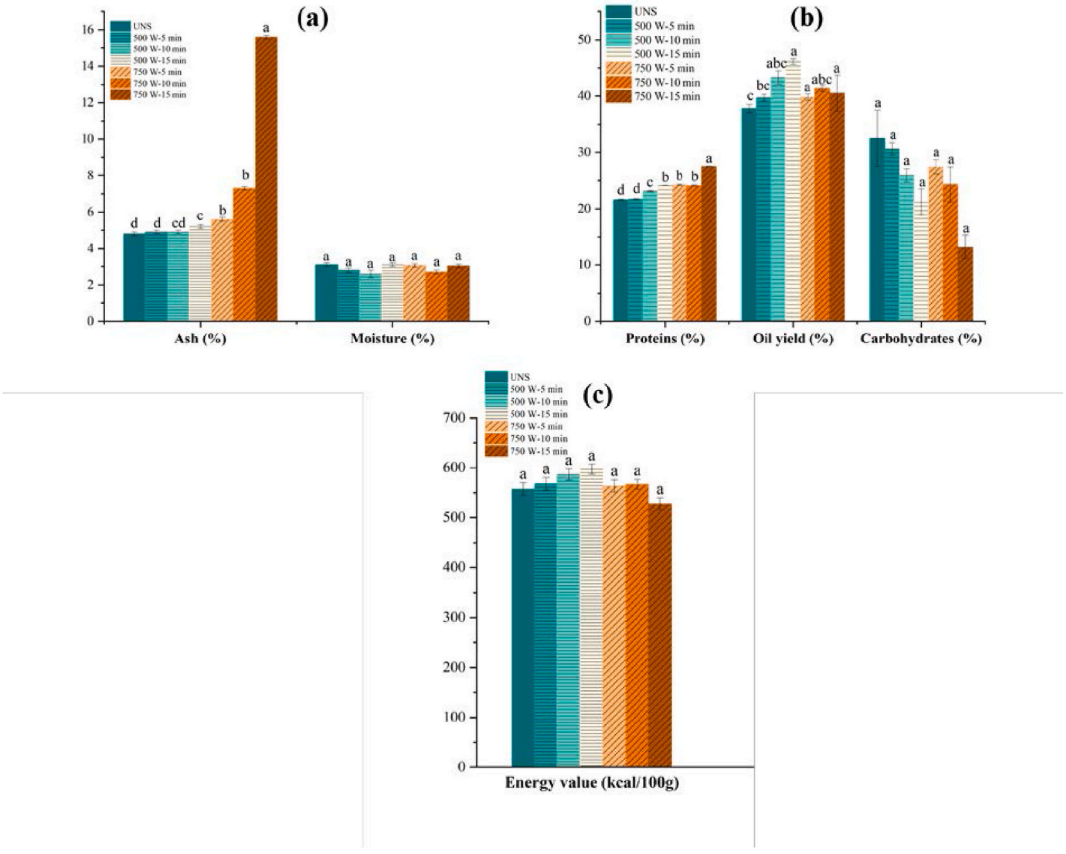


**Fig. 1.** Effect of microwave roasting on Ash (a), moisture (a), proteins(b), oil yield(b), carbohydrates (b), and energy value (c) in black cumin seeds. *UNS: Unroasted Nigella Seeds; data values of each parameter with the same alphabetic exponent within a row do not vary significantly (P < 0.05).*

### 3.2. Proximate composition

The microwave treatment effect on the proximate composition of black cumin seeds are shown in Fig. 1. Roasting treatment has significantly ($P < 0.05$) affected the biochemical content of the seeds in different manner depending on the roasting conditions (Fig. 1).

The ash content increased from 4.8 ± 0.1 % for unroasted cumin seeds to 5.2 ± 0.1 % and 15.6 ± 0.1 % for roasted seeds at 500 W and 750 W for 15 min of treatment, respectively (Fig. 1,(a)).

Furthermore, the roasting treatment significantly improved the protein content. The initial value in unroasted black seeds was 21.6 ± 0.1 %, but it increased to 27.5 ± 0.03 % at 750 W for 15 min. This increase is primarily due to the concentration effect caused by the quick evaporation of moisture during the roasting process. As water content decreases, the concentration of proteins and other solid components becomes more pronounced. Additionally, the microwave roasting process may cause structural changes in the seeds that enhance the stability and availability of proteins. The heating effect can also lead to partial denaturation of proteins, which might make them more soluble or more readily measurable. These combined effects result in a higher apparent protein content in the roasted seeds compared to their unroasted counterparts.

On the other hand, the roasting process did not produce significant changes in the moisture content which was 3.1 ± 0.1 % for the unroasted seeds and 3.03 ± 0.1 % for the roasted seed at 750 W for 15 min. Harhar et al. (2011) [45] showed that the water content decreased after roasting argan kernels. This low moisture content could influence various processes related to proteins including a decrease in the solubility of proteins, an increase in the content of proteins linked to cell walls and to lignin, or to the proteins of the Maillard reaction [46].

Fig. 1,(b) Fig. 1 also displays the oil yields of black cumin seeds, both roasted and unroasted. The oil yield from raw NS was 37.8 ± 0.7 %. a value of 20.13 % oil yield from raw NS was reported by Acar et al. [47]. Another study showed that Twenty-three genotypes of nigella obtained from India showed oil yields ranging from 14.7 to 27.0 % [48]. The obtained data showed that the oil content increased with the duration and the roasting power, 39.7 ± 0.6 %, 43.3 ± 1.2 %, and 46.1 ± 0.5 % at 500 W for 5 min, 10 min, and 15 min, respectively. Similarly, at 750 W the content was 39.8 ± 0.6 % for 5 min and 41.4 ± 0.6 % for 10 min. The microwave causes the evaporation of water in the plant cell which increases porousness and permeability, as well as the pressure in the internal environment leading to the decomposition of the cell, rupture of the membrane, resulting in an improvement of the oil extraction efficiency [49]. Two other studies showed that microwave roasting enhanced the oil yield of flax seeds and apricot kernels [1,50]. However, a drop in the oil content was observed during the maximum roasting time (750 W for 15 min). In fact, the bioactive components may degrade under excessive microwave power and time, which will lower the extraction yield [51].

Carbohydrates, fiber, starches, and sugars are essential food nutrients that our bodies convert into glucose to give us the energy we need to function [52]. The carbohydrates content of unroasted black cumin seed was 32.5 ± 5.0 %, which concurred with the value reported by Al-Jassir [53]. This initial carbohydrate content of black cumin seeds decreased after 15min of roasting to 21.2 ± 2.3 % for 500 W and 13.2 ± 2.1 % for 750 W. The results are in harmony with Makinde et al. who also reported a decrease in carbohydrates content after roasting treatment [54]. This loss of carbohydrates after roasting could be attributed to the role of sugar as a precursor in the production of roasted nigella aroma, where it provides a carbon source for the production of aroma compounds following the Maillard reaction [55].

Differences were also recorded by comparing energy values (Fig. 1,(c)). Thus, for unroasted cumin seeds, the energy value was 557.3 ± 12.6 kcal/100 g, while at 500 W for 5, 10, and 15 min the values increased as a function of time. At 750 W the energy value also increased until 15 min where the value decreased (527.8 ± 12 kcal/100 g). Taking into consideration the formula of the calculation of the energy value, we can see that the oil content is multiplied by the largest factor. Therefore, the change of the energy value will be mainly influenced by the oil content, so the energy value has the same behavior as the fat content, comparable outcomes were stated by Rękas et al. [56].

**Table 2**
Effect of microwave roasting on relative fatty acid composition of black cumin seed oils (g/100g).

| | UNS | 500 W | | | 750 W | | |
|---|---|---|---|---|---|---|---|
| | | 5 min | 10 min | 15 min | 5 min | 10 min | 15 min |
| **C14 : 0** | 0.18 ± 0.01$^{a}$ | 0.18 ± 0.01$^{a}$ | 0.18 ± 0.01$^{a}$ | 0.18 ± 0.01$^{a}$ | 0.18 ± 0.01$^{a}$ | 0.19 ± 0.01$^{a}$ | 0.19 ± 0.01$^{a}$ |
| **C16 : 0** | 12.7 ± 0.1 $^{ab}$ | 12.5 ± 0.1$^{b}$ | 12.4 ± 0.1$^{b}$ | 12.5 ± 0.1$^{b}$ | 12.5 ± 0.1$^{b}$ | 12.6 ± 0.1 $^{ab}$ | 13.1 ± 0.1$^{a}$ |
| **C16 : 1** | 0.19 ± 0.01$^{a}$ | 0.22 ± 0.01$^{a}$ | 0.21 ± 0.01$^{a}$ | 0.20 ± 0.01$^{a}$ | 0.22 ± 0.01$^{a}$ | 0.22 ± 0.01$^{a}$ | 0.22 ± 0.01$^{a}$ |
| **C18 : 0** | 3.09 ± 0.01$^{a}$ | 3.02 ± 0.01$^{a}$ | 2.99 ± 0.01$^{a}$ | 2.94 ± 0.01$^{a}$ | 2.99 ± 0.01$^{a}$ | 2.95 ± 0.01$^{a}$ | 2.73 ± 0.01$^{a}$ |
| **C18 : 1** | 22.6 ± 0.1$^{b}$ | 22.6 ± 0.1$^{b}$ | 22.6 ± 0.1$^{b}$ | 22.6 ± 0.1$^{b}$ | 22.6 ± 0.1$^{b}$ | 22.8 ± 0.1$^{b}$ | 23.4 ± 0.1$^{a}$ |
| **C18 : 2** | 60.4 ± 0.1$^{a}$ | 60.7 ± 0.1$^{a}$ | 60.7 ± 0.1$^{a}$ | 60.8 ± 0.1$^{a}$ | 60.6 ± 0.1$^{a}$ | 60.5 ± 0.1$^{a}$ | 59.7 ± 0.1$^{b}$ |
| **C18 : 3** | 0.20 ± 0.01$^{a}$ | 0.21 ± 0.01$^{a}$ | 0.21 ± 0.01$^{a}$ | 0.20 ± 0.01$^{a}$ | 0.22 ± 0.01$^{a}$ | 0.19 ± 0.01$^{a}$ | 0.14 ± 0.01$^{a}$ |
| **C20 : 0** | 0.1 ± 0.01$^{a}$ | 0.1 ± 0.01$^{a}$ | 0.10 ± 0.01$^{a}$ | 0.1 ± 0.01$^{a}$ | 0.10 ± 0.01$^{a}$ | 0.1 ± 0.01$^{a}$ | 0.1 ± 0.01$^{a}$ |
| **C20 : 1** | 0.25 ± 0.01$^{a}$ | 0.27 ± 0.01$^{a}$ | 0.29 ± 0.01$^{a}$ | 0.23 ± 0.01$^{a}$ | 0.29 ± 0.01$^{a}$ | 0.20 ± 0.01$^{a}$ | 0.19 ± 0.01$^{a}$ |
| **SFA** | 16.1 ± 0.1$^{a}$ | 15.9 ± 0.1 $^{ab}$ | 15.8 ± 0.1 $^{ab}$ | 15.7 ± 0.1 $^{ab}$ | 15.5 ± 0.1$^{b}$ | 15.9 ± 0.1 $^{ab}$ | 16.1 ± 0.1$^{a}$ |
| **MUFA** | 23.07 ± 0.1$^{b}$ | 23.1 ± 0.1$^{b}$ | 23.1 ± 0.1$^{b}$ | 23.1 ± 0.1$^{b}$ | 23.2 ± 0.1$^{b}$ | 23.3 ± 0.1$^{b}$ | 23.9 ± 0.1$^{a}$ |
| **PUFA** | 60.6 ± 0.1$^{a}$ | 60.9 ± 0.1$^{a}$ | 60.9 ± 0.1$^{a}$ | 61.03 ± 0.1$^{a}$ | 60.8 ± 0.1$^{a}$ | 60.7 ± 0.1$^{a}$ | 59.8 ± 0.1$^{b}$ |

UNS: Unroasted Nigella Seeds; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids. All values are mean ± standard deviation of three replicates. Different letters within a column indicate significant statistical differences ($P < 0.05$).

### 3.3. Fatty acid composition

The fatty acid composition of unroasted and roasted black cumin seed oils is shown in Table 2. The results expose that the major unsaturated fatty acids are linoleic acid C18:2 and oleic acid C18:1. The major saturated FAs were palmitic C16:0 and stearic C18:0 acids, whereas, myristic C14:0 and arachidic C20:0 acids were the least abundant fatty acids in black seed oils (Table 2).

These outcomes are consistent with the results of Amin et al. and Farhan et al. [57,58], while Yimer et al. reported marginally higher values [59]. The fatty acid profile of black cumin seed oil is slightly affected by microwave roasting for 5–15 min and power of 500 and 750 W. PUFAs are the most abundant with values over 60 g/100 g. However, after 15 min of roasting at 750 W, the amount diminished to 59.8 ± 0.1 g/100g. This slight decrease may be due to the oxidative and non-oxidative degradation of SFAs and PUFAs caused by the heat treatment [60]. On the other hand, there was no discernible change in the fatty acid composition of cactus seeds (*Opuntia ficus-indica* L.), roasted at 110 ± 5 °C for 10, 20, 30 and 40 min using a roaster with continuous mixing of the material [61]. When roasting with a microwave, the relative content of polyunsaturated fatty acids (PUFAs) in unroasted pumpkin seeds decreased to 84.7 %, and SFAs increased to 119.5 % after 9 h of heating. In contrast, for samples roasted for 12 min, PUFAs decreased to 97.0 %, and SFAs increased to 102.6 % after the same 9 h heating period [62].

### 3.4. Sterol composition

Important information regarding the quality and the identity of the oil under investigation is revealed by the study of the sterols. The total sterol content of unroasted black cumin seed oil extracted by mechanical press was 1399.6 ± 0.5 mg/100g (Table 3).

As reported in previous studies [63–65], β-sitosterol, stigmasterol, and campesterol have been found to be the main sterols in unroasted cumin seed oil, representing 52.5 ± 0.5, 17.3 ± 0.5, and 11.8 ± 0.5 g/100g, respectively. The minor sterols are cholesterol, Δ-5-avenasterol, Δ-7-stigmasterol, and Δ-7-avenasterol. As observed, the rate of stigmasterol decreased as the power and time of roasting increased, while the amounts of β-sitosterol increased in relation to the same factors. These changes are moderately significant, with the total sterol content augmenting from 1399.6 ± 0.5 mg/kg, in unroasted seeds, to 1512.3 ± 0.5 mg/kg (15 min/750 W) (Table 3). These results are similar to those reposted by Nounah et al. that revealed a change in sterol composition after the roasting of cactus [66]. In an additional study, after 20 min in an electric oven, a drop in the phytosterol composition of sesame oil was reported [67].

### 3.5. Total tocopherols, total polyphenols, and antioxidant activity

The value of total tocopherols for unroasted black cumin seeds was 225.25 ± 0.1 mg/100g (Fig. 2,(a)). Another work found a value of 247 mg/100g in total tocopherols, this variance can be explained by the difference in the geographic source of cumin seeds [68]. The results of the present study show that roasting could have an effect on the tocopherol content, this effect changes according to the roasting parameters. For example, at 500 W for 10 min the tocopherol content decreased to 194.46 ± 0.1 mg/100g, while at 750 W for 10 min the content increased to 283.59 ± 0.1 mg/100g. While some studies found a decrease in tocopherol content after roasting [69], other experiments revealed that roasting increased the tocopherol content using both conventional or microwave roasting methods [58,59]. The total phenolic compound of unroasted and roasted black cumin seeds are presented in Fig. 2,(b). Our results disclosed that roasting treatment significantly increases the polyphenol content, from 22.2 mg GAE/g for unroasted black cumin seeds to 165.5 mg GAE/g for the roasted seeds at 750 W after 5min. The explanation of this increase is that microwave roasting can destroy the cell structure of the cladding and release bound phenolic compounds, with the increase of the polyphenol levels [21]. Previous studies have also reported similar results, using a domestic electric oven as a roasting tool and setting three exposure times (10, 15 and 20 min) and three temperatures (180, 200 and 220 °C), the phenolic content of Iranian sesame seeds (*Sesamum indicum*) increased significantly as a function of roasting temperature [69]. From the outcomes, it was concluded that the optimum temperature and roasting time to achieve the highest total phenolic content was 750 W for 5 min.

The evaluated antioxidant activity for unroasted black seed oil was recorded as 45.01 ± 0.8 %. After 15 min of roasting at 500 W this value increased to 56.1 ± 1.5 % (Fig. 2,(b)). These results are in agreement with other studies that also sought to increase the

**Table 3**
Effect of microwave roasting on sterol composition of black cumin seeds (g/100g).

| | UNS | 500 W | | | 750 W | | |
|---|---|---|---|---|---|---|---|
| | | 5 min | 10 min | 15 min | 5 min | 10 min | 15 min |
| **Cholesterol** | $1.2 \pm 0.05^{a}$ | $1.07 \pm 0.05^{a}$ | $1.02 \pm 0.05^{a}$ | $0.9 \pm 0.05^{a}$ | $1.2 \pm 0.05^{a}$ | $2.0 \pm 0.05^{a}$ | $1.09 \pm 0.05^{a}$ |
| **Campesterol** | $11.8 \pm 0.5^{a}$ | $12.0 \pm 0.5^{a}$ | $12.4 \pm 0.5^{a}$ | $11.3 \pm 0.5^{a}$ | $10.9 \pm 0.5^{a}$ | $11.2 \pm 0.5^{a}$ | $11.3 \pm 0.5^{a}$ |
| **Stigmasterol** | $17.3 \pm 0.5^{a}$ | $17.3 \pm 0.5^{a}$ | $17.05 \pm 0.5^{ab}$ | $14.7 \pm 0.5^{abc}$ | $15.5 \pm 0.5^{abc}$ | $14.1 \pm 0.5^{bc}$ | $13.1 \pm 0.5^{bc}$ |
| **β-Sitosterol** | $52.5 \pm 0.5^{a}$ | $52.4 \pm 0.5^{a}$ | $53.3 \pm 0.5^{a}$ | $53.4 \pm 0.5^{a}$ | $48.7 \pm 0.5^{b}$ | $53.08 \pm 0.5^{a}$ | $55.7 \pm 0.5^{a}$ |
| **Δ-5-Avenasterol** | $5.1 \pm 0.5^{ab}$ | $7.1 \pm 0.5^{b}$ | $5.5 \pm 0.5^{b}$ | $9.2 \pm 0.5^{a}$ | $9.4 \pm 0.5^{a}$ | $8.3 \pm 0.5^{ab}$ | $7.9 \pm 0.5^{ab}$ |
| **Δ-7-Stigmasterol** | $0.3 \pm 0.05^{a}$ | $0.6 \pm 0.05^{a}$ | $0.7 \pm 0.05^{a}$ | $0.5 \pm 0.05^{a}$ | $0.8 \pm 0.05^{a}$ | $1.02 \pm 0.05^{a}$ | $0.6 \pm 0.05^{a}$ |
| **Δ-7-Avenasterol** | $1.5 \pm 0.05^{a}$ | $1.5 \pm 0.05^{a}$ | $1.03 \pm 0.05^{a}$ | $1.5 \pm 0.05^{a}$ | $2.1 \pm 0.05^{a}$ | $1.4 \pm 0.05^{a}$ | $1.6 \pm 0.05^{a}$ |
| **Others** | $11.3 \pm 0.5^{a}$ | $7.5 \pm 0.5^{c}$ | $8.7 \pm 0.5^{abc}$ | $8.1 \pm 0.5^{bc}$ | $11.06 \pm 0.5^{ab}$ | $8.6 \pm 0.5^{abc}$ | $8.3 \pm 0.5^{abc}$ |
| **Total mg/kg** | $1399.6 \pm 0.5^{f}$ | $1559.1 \pm 0^{d}$ | $1265.3 \pm 0.5^{g}$ | $2979 \pm 0.5^{a}$ | $1606.7 \pm 0.5^{b}$ | $1596.4 \pm 0.5^{c}$ | $1512.3 \pm 0.5^{e}$ |

UNS: Unroasted Nigella Seeds; data values with the same alphabetic exponent within a row do not vary significantly ($P < 0.05$).

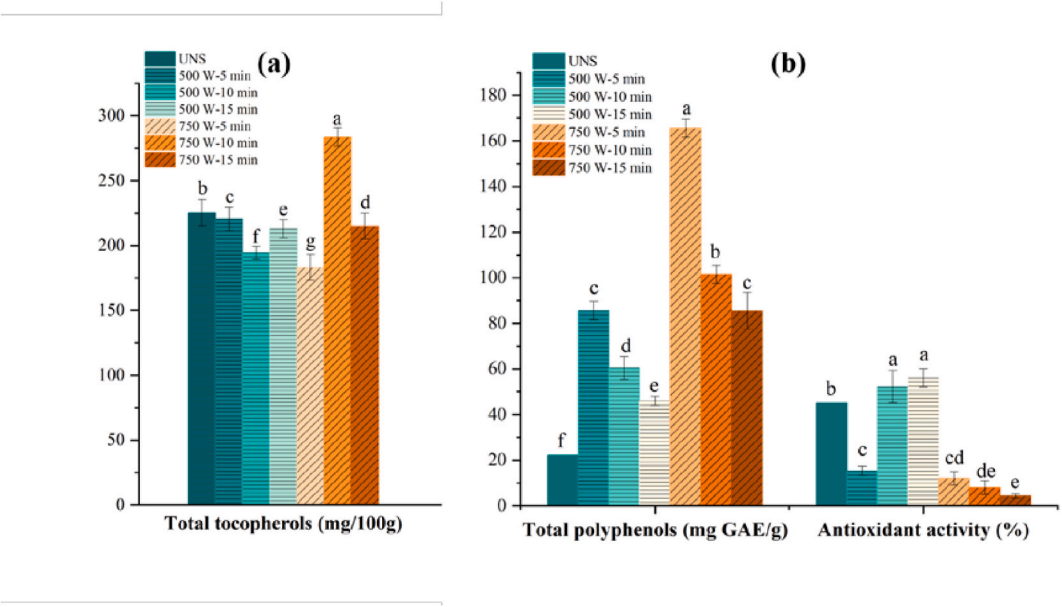


**Fig. 2.** Effect of microwave roasting on total tocopherols (a), total polyphenols (b), and antioxidant activity (b) of black cumin seeds. *UNS: Unroasted Nigella Seeds; data values of each parameter with the same alphabetic exponent within a row do not vary significantly ($P < 0.05$).*

antioxidant capacity of the matrices studied through roasting [70–72]. On the other hand, at 750 W the antioxidant activity decreased in a remarkable way to reach 4.3 ± 0.9 % after 15 min. This decrease in the antioxidant capacity after roasting has also been found by Alkaltham et al. (2020) [73] and Haile et al. (2020) [74]. This variation can be interpreted by the degradation of components, such as polyphenols, that have the ability to scavenge free radicals after exposure to a very high level of heat. Comparatively, sesame seeds subjected to microwave roasting at 720 W for 15 min showed the highest antioxidant activity and total phenols content [75].

### 3.6. Quality indices and pigments

#### 3.6.1. Acidity value

The AVs of black seed oil presented in Table 4 show an increase as a function of time and roasting power. The initial value was 3.3 ± 0.01 g/100g, after microwave roasting, the acidity of the oil gradually increases, reaching higher values of 4.6 ± 0.02 g/100g and 5.6 ± 0.01 g/100g, after 15 min of roasting at 500 W and 750 W, respectively (Table 4). This may be explained by the fact that high temperatures cause thermal degradation of the oil, breaking down triglycerides into free fatty acids, which directly increases acidity values [76,77]. Additionally, the uneven heating typical of microwave roasting accelerates oxidation, leading to the formation of acidic by-products. Our results are in agreement with those of Rękas et al. [78], who examined the effect of roasting on rapeseed.

#### 3.6.2. Peroxide value

Peroxide value (PV) is a marker for the early stages of oxidative changes [79]. A remarkable increase in the PV of black cumin seed oils was revealed after roasting (Table 4). Indeed, the initial value was 30.4 ± 0.2 mEq ($O_2$)/kg, it reaches maximum values of 40.8 ± 1.0 and 50.4 ± 0.6 mEq ($O_2$)/kg after 15 min of roasting at 500 W and 750 W, respectively. The increase in the PV of microwave roasted black cumin oils could be due to free radical attack on unsaturated fatty acids resulting in the formation of a higher content of primary oxidation products under the effect of temperature, the high microwave energy accelerates the lipid oxidation process by providing sufficient thermal energy to initiate and propagate the formation of peroxides [56]. Our results are in agreement with those

**Table 4**
Effect of microwave roasting on quality indices and pigments of black cumin seeds.

| | UNS | 500 W | | | 750 W | | |
|---|---|---|---|---|---|---|---|
| | | 5 min | 10 min | 15 min | 5 min | 10 min | 15 min |
| **AV (g/100g)** | $3.3 \pm 0.01^{d}$ | $3.3 \pm 0.1^{d}$ | $3.9 \pm 0.01^{c}$ | $4.6 \pm 0.02^{b}$ | $3.3 \pm 0.01^{d}$ | $4.2 \pm 0.06^{c}$ | $5.6 \pm 0.01^{a}$ |
| **PV (mEq($O_2$)/kg)** | $30.4 \pm 0.2^{d}$ | $38.7 \pm 0.5^{c}$ | $39.4 \pm 0.6^{c}$ | $40.8 \pm 1.0^{c}$ | $45.5 \pm 1.0^{b}$ | $48.2 \pm 0.3^{ab}$ | $50.4 \pm 0.6^{a}$ |
| **Absorption K232 (%)** | $3.2 \pm 0.3^{e}$ | $7.2 \pm 0.04^{d}$ | $10.8 \pm 0.2^{c}$ | $12.8 \pm 0.4^{b}$ | $6.2 \pm 0.1^{d}$ | $13.4 \pm 0.3^{b}$ | $17.4 \pm 0.30^{a}$ |
| **Para-anisidine (AV)** | $13.1 \pm 0.01^{d}$ | $11.4 \pm 0.01^{e}$ | $13.6 \pm 0.01^{d}$ | $19.04 \pm 0.04^{c}$ | $9.8 \pm 0.01^{f}$ | $27.8 \pm 0.02^{b}$ | $29.2 \pm 0.02^{a}$ |
| **Carotenoids (mg/100g)** | $6.7 \pm 0.01^{f}$ | $7.3 \pm 0.01^{e}$ | $7.4 \pm 0.01^{e}$ | $11.05 \pm 0.04^{c}$ | $7.6 \pm 0.01^{d}$ | $15.4 \pm 0.02^{b}$ | $17.2 \pm 0.02^{a}$ |
| **Chlorophylls (mg/100g)** | $2.7 \pm 0.01^{f}$ | $4.9 \pm 0.01^{d}$ | $5.02 \pm 0.01^{d}$ | $5.2 \pm 0.04^{c}$ | $3.4 \pm 0.01^{e}$ | $18.6 \pm 0.02^{b}$ | $19.5 \pm 0.02^{a}$ |

UNS: Unroasted Nigella Seeds; data values with the same alphabetic exponent within a row do not vary significantly ($P < 0.05$).

of Muangrat et al. [80], Mohammed et al. [81], who studied the oxidative stability effect after microwave roasting on different plant matrices.

### 3.6.3. Specific extinction K232

K232 value is typically another quality measure of the primary oxidative state of oils [82], which measure conjugated dienes and trienes (Table 4). The specific extinction of unroasted black seed oil was 3.2 ± 0.3. However, after 15 min of roasting at 500 W and 750 W, it reached values of 12.8 ± 0.4 and 17.4 ± 0.3, respectively. Besides the peroxide value, these results suggest that the primary oxidation products increase with increasing power and exposure time. During roasting, triglycerides in the oil undergo oxidative cleavage, producing hydroperoxides as primary oxidation products. Suri et al. studied the effects of infrared and dry air roasting on the oxidative stability, fatty acid content, and other chemical parameters of black seed oil, and their findings are consistent with ours [83].

### 3.6.4. Para-anisidine value

In addition to assessing peroxide value, which provides an overview of primary oxidation products, para-anisidine value is a parameter used to measure secondary oxidation products [84]. The initial value of the p-AV in the black cumin seed oil was 13.1 ± 0.01. After roasting, a decrease was observed after 5 min at 500 W and 750 W, values of 11.4 ± 0.01 and 9.8 ± 0.01, respectively (Table 4). A slight increase was observed for roasting at 500 W and 750 W after 10 min, while remarkable differences were detected for both 500 W and 750 W, after 15 min, showing values of 19.04 ± 0.04 and 29.2 ± 0.02, respectively. An elevated para-anisidine value in black cumin oil roasted at high temperatures signifies heightened fatty acid oxidation, largely driven by heat-sensitive hydro--peroxides [85]. These compounds decompose under heat, forming reactive radicals and products like aldehydes and ketones, indicating increased oxidative degradation in the oil. by evaluating the para-anisidine index of sesame seed oil subjected to a conventional oven roasting treatment Mohamed Ahmed et al. (2021) also found that the highest values of this parameter are presented by the oil extracted from seeds subjected to the highest temperature (220 °C) [60].

### 3.6.5. Pigment content

Chlorophylls and carotenoids are prevalent pigments responsible for the vibrant colors found in numerous vegetables and fruits [84]. The carotenoid and chlorophyll contents of black seed oils increased with the roasting time of the seeds. Indeed, the initial carotenoid content was 6.7 ± 0.01 mg/100g and it significantly increased to a value of 17.2 ± 0.02 mg/100g at 750 W for 15 min of roasting (Table 4). Likewise, the initial chlorophyll value of 2.7 ± 0.01 mg/100g increased after roasting to a maximum value of 19.5 ± 0.02 mg/100g. By denaturing the proteins, the heat treatment disintegrated the complexes between bound proteins and pigments, and this could be the mechanism behind the increased pigment levels in the oils. Alternatively, it can also be explained with the fact that roasted seeds develop more abundant pigments mainly due to the Maillard and caramelization reactions that occur during the roasting process [85]. These chemical reactions lead to the formation of complex colour compounds that contribute to the range of colors seen in roasted seeds, from golden brown to dark brown. An increase in chlorophyll and β-carotene levels is also reported by Różańska et al. (2019) by subjecting different oilseeds to conventional roasting in an oven set at 140 °C for 15 min, these obtained results are also in agreement with those of Rabadán et al. (2018). who studied the effect of oven roasting pretreatments on the physical parameters, oxidative stability, and pigment composition of pistachio oil [86,87].

In another study where pumpkin seeds were roasted using microwaves, it was also found that oxidation indices such as free fatty acid, peroxide value, para-anisidine value, specific extinctions and the thiobarbituric acid index (TBA) of the oils increased significantly, and the increases were found to be significantly higher ($p < 0.05$) in the unroasted seed oil compared with the roasted seed oil [62].

## 4. Conclusions

This study focused on determining the antioxidant activities of *Nigella sativa* seed oils and analysing the microwave processing technique effect on the physico-chemical properties of the black cumin seeds and oils from plants cultivated in Morocco. Oil yield, mineral content, total polyphenol content, tocopherols content, and total antioxidant capacity were all significantly affected by roasting. Thus, the oil yield increased after the roasting process. Analyses of the antioxidant compounds revealed that the optimal level of total polyphenols was obtained at 750 W for 5 min. In addition, a significant decline was noted in the total antioxidant capacity for roasted black cumin seeds associated in part with a decrease in polyphenol content. Nevertheless, the analysis of treated seeds revealed no change in the profiles of fatty acids. These findings indicate that the preparation of black cumin seed oils should be handled judiciously, with post-extraction measures able to protect the antioxidant and anti-inflammatory capacities, prevent cellular senescence, and delay aging processes.

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## Data availability statement

Not applicable.

## CRediT authorship contribution statement

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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