Evolution of Tocopherol Content, Polyphenol Profile, Fatty Acid Profile and Antioxidant Capacity of Fixed Oils of *Nigella Sativa* L. Seeds During Roasting Time

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Abstract: Nigella *sativa* L. is a plant widely used in traditional medicine, and according to our knowledge no study was made on the effect of roasting on the chemical composition and the antioxidant activity of the fixed oils of *N. sativa* L. seeds. Therefore, we found it useful to investigate this parameter. The seeds are roasted for varying durations (0-40 min) at 180°C. The phenolic content, the fatty acid composition, and the tocopherol composition were determined using chromatographic methods (HPLC and GC). For the antioxidant activity, we used two chemical tests: DPPH (2, 2-diphenyl-1-picrylhydrazyl) test and the β-carotene bleaching test. The maximum chemical content of seed oils was recorded after 15 min of roasting. On the other hand, the concentration of tocopherols decreased over time. The antioxidant activity of treated seed oils was also determined *in vitro* using DPPH free radical scavenging activity and the β-carotene bleaching tests. In both tests, the highest results were obtained for 15 min roasted sample. The roasting process considerably reduces the content of tocopherols in the fixed oils of the seeds of *N. sativa*, nevertheless the roasting has a beneficial effect on the content of polyphenols and the antioxidant activity

Keywords: Fixed oils, *Nigella sativa* L., Roasting, Phenolic acids, Antioxidant activity, Tocopherols.

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Introduction

Nigella sativa L. is among the most widely used medicinal plants in the world. It is considered as a medicine for digestive and liver disorders, as well as for chronic headaches and migraines. In dermatology, the seeds are used to treat alopecia, eczema and acne due to their anthelmintic and anti-infectious properties[1]. Numerous studies on the phytochemistry and bioactivity of *N. sativa* L. have confirmed these properties, which are mainly due to the fixed oils and polyphenols it contains. The fixed oils from seeds of *N. sativa* L. have many pharmacological properties and can be considered as antioxidant, anti-inflammatory, immunomodulating, anti-tumor, and anti-diabetic agent and play a significant role in the cardiovascular and gastrointestinal systems[2]. Abu Ali Al-Hussein Ibn Abdallah Ibn Sina, known as Avicenna (980-1037), discussed *N. sativa* L. in his book "Kitab Al-Shifâ" or "The Book of the Healing of the Soul". He advised roasting the seeds before use[3]. Heat treatment changes the nutritional profile of the seeds increasing antioxidant activity and reducing anti-nutritional factors such as tannins[4]. Besides, roasting also improves the digestibility and shelf life of seeds[5], so we found it useful to investigate the impact of heat treatment on composition and biological activity of the fixed oils of *N. sativa* L. seeds.

Materials And Methods

Chemical and reagents

All solvents and reagents were of the highest purity required for each application. Hexane, acetone, methanol, ethyl acetate, orthophosphoric acid, aluminum chloride, ammonium sulfate, sodium sulfate, vitamin E, linoleic acid, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH, 98%), hydroxytoluene-butyl (BHT), hydroxyanisolebutyl alcohol (BHA), β-sitosterol and the standards (gallic acid, catechin, vanillic acid, epicatechin, coumaric acid, apigenin, rutin, quercetin, kaempferol) were supplied from Sigma-Aldrich (SARL Prochima-Sigma, Tlemcen, Algeria). β-carotene and Tween 20 were purchased from Flucka (Buchs, Germany).

Plant material

Seeds constitute the part of the plant used in this study. They were cultured in the Saudi Arabia. The seeds were ground in a blender to obtain a powder.

Samples preparation.

100 g of the *N. sativa* L. seeds were spread on an aluminum tray to a thickness of 1 cm and placed in an oven set at 180°C. Samples treated for 5, 10, 15, 20, 30 and 40 min were immediately cooled to room temperature and stored at 4°C in plastic bags. Untreated samples were also stored under the same conditions. The oils were extracted from the seeds by hot solvent (hexane) using Soxhlet and stored in glass containers at 4°C until used to determine their chemical composition (by chromatographic and colorimetric methods) and antioxidant activity.

Fatty acid analysis.

Forty milligrams of the fixed oils were methylated with 3 mL of 60 g/L HCl in methanol at 75-80°C for 2 hr. FAME was extracted with 2 mL hexane and dried with sodium sulfate[6]. 1 microliter of FAME was analyzed with an Agilent 7890 series gas chromatograph (Agilent Company) equipped with a flame ioni-

zation detector and 7683B autoinjector. A DB23 fused silica capillary column (60 m, 0.25 mm diameter, 0.25 0.25 µm film thickness; J& W Scientific, Folsom, CA) was used. The furnace temperature was programmed as follows: 140°C for 5 min, increased to 240°C at 3 °C/min, and maintained at 240°C for 10 min. Injector and detector temperatures were maintained at 250°C, the carrier gas was helium, the flow rate was 30 mL/min and the split ratio was 1/30. FAME identification was based on retention times relative to those of the standard FAME mixture[7]. The results were expressed in percent of the peak area without any correction. Fatty acid analysis was performed in triplicate for each sample.

Sterol determination by ultraviolet-visible (UV-Vis).

It is a spectrophotometric absorption according to the Liebermann-Burchard test[8] based on a specific color reaction of the 3 β-hydroxysteroïdes sterols having a double bond in position 5-6. The sterols form a stable complex with acetic anhydride in acidic medium, which absorbs in the visible at 550 nm. The Liebermann spectral reagent consists of 60 mL acetic anhydride and 10 mL concentrated sulfuric acid and 30 mL acetic acid. Based on a chloroformic solution of cholesterol (concentration of 1 g/l), a series of solutions was prepared in order to draw a calibration curve linking the absorbance to the concentration. We take 1 mL of each solution and add 2 mL of Liebermann's reagent and allow the coloration to develop and stabilize for 25 min. The absorbance at 550 nm of each solution is measured and the calibration curve is obtained. in the same way, the sample is treated and the sterol (β-sitosterol) content is determined from the calibration curve.

Analysis of tocopherols.

Analyses were carried out by using a Shimadzu Prominence HPLC system (Columbia, MD) consisting of quaternary pump, autosampler, in-line degasser, column oven and fluorescence (extinction: 290 nm, emission: 330) detector. Normal phase chromatographic separation was achieved with a NH_2 column (5.0 µm, 4.60 × 250 mm, Inertsil) at 30°C. Oil samples were diluted ten times in mobile phase (4% 2-propanol in hexane) and 20 µL of this solution was injected on to the NH_2 column. Elution was programmed as isocratic flow of 4% 2-propanol in hexane for 30 min. Flow rate was set to 1 mL/min and the eluate was monitored at 290 nm. Tocopherols (α, β, **γ**, δ) were quantified based on peak areas compared with external standards. For each sample, analyses were carried out in triplicate, and average values were reported [9].

Polyphenol extraction from fixed oils.

To extract the phenolic compounds, 0.5 g of oil was weighed into a glass test tube and 4 mL of methanol: water (80:20, v/v) mixture was added. The tube was vortexed for 1 min and the aqueous layer was collected after centrifugation at 3000 rpm for 5 min. The procedure was repeated three times and the aqueous extracts were combined. To remove the residual oil, the extracts were washed twice with 2 mL of hexane. After removal of methanol from the extract, the remaining aqueous phase was then washed several times with the same volume of ethyl acetate in the presence of a combination of two aqueous solutions of 2% ammonium sulfate and 2% orthophosphoric acid. The organic extract thus obtained is evaporated to dryness after drying over anhydride sodium sulfate [7].The phenolic extract thus obtained has a viscous yellowish-brown appearance. The residue is taken up in 10 mL of pure methanol and kept at 5°C giving the purified phenolic extract.

Total phenolic content.

The determination of total phenols was carried out by a method adapted from Singleton and Ross[10]. In basic medium, the Folin-Ciocalteu reagent oxidizes the oxidizable groups of the polyphenolic compounds present in the sample. The blue-colored reduction products show a maximum absorption intensity proportional to the quantity of polyphenols present in the sample. The mass concentration of the constituents used in the preparation of the reagents was optimized to obtain the most possible linear analytical response while respecting the reagents/total polyphenols ratio. To perform this assay, in short, 1 mL of Folin-Ciocalteu reagent diluted to ten and 1 mL of 2% Na_2CO_3 was added to 1 mL of oil extract suitably diluted in absolute ethanol. The absorbance at 760 nm was measured after 1 hour incubation at room temperature and the results are expressed as gallic acid equivalent (GAE).

Individual phenolic identification by HPLC.

The qualitative and quantitative analysis of the phenolic compound of the methanol extract of the fixed oils of the seeds of *N. sativa* L. was performed on a Varian analytical HPLC system model Pro Star 230 (Varian Associates, Walnut Creek, California, USA) equipped with a ternary pump (model Q2 Pro Star 230) and a photodiode array detector (model Prostar 335). HPLC separation of the active compounds was performed on a C-18 reversed-phase HPLC column (Zorbax, 30 cm x 4.6 mm, 5 pm particle size). Elution was performed using a binary system consisting of solvent A (acetonitrile) and solvent B (2% glacial acetic acid solution (pH = 2.6)) supplied by Sigma-Aldrich (Germany). The gradient program was used as follows: 0-5 min: 5% A and 95% B, 25-30 min: 35% A and 65% B, 35-45 min: 70% A and 30% B at a flow rate of 0.9 mL/min. The injection volume was 20 µL and detection was performed at 280 and 360 nm. The identification of peaks was confirmed by comparing their retention times with pure standards and quantified by comparing the peak areas in the chromatograms of the samples with those of the standards (gallic acid, catechin, vanillic acid, epicatechin, coumaric acid, apigenin, rutin, quercetin, kaempferol) under the same conditions[11]. All measurements were performed in triplicate, expressing the results as mean values ± standard deviation of micrograms of phenolic compound/g fixed oils of seeds of *N. sativa* L.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) test.

The reduction of the free radical (2, 2-diphenyl-1-picrylhydrazyl) DPPH by an antioxidant can be monitored by UV-Vis spectrophotometry, measuring the decrease in absorbance caused by the presence of the extracts. The scavenging capacity of the DPPH radical by the extracts was determined according to the method of Espín et al.[12]. 100 µL of the sample at various concentrations is added to 2.9 mL of the DPPH solution (0.033 g/L). A negative control was prepared by mixing 100 µL of methanol with 2.9 mL of the DPPH solution. The resulting absorbance was measured with a UV-Vis spectrophotometer (OPTIZEN 2120UV, Korea) at 520 nm after 30 min incubation in darkness and at room temperature. We used BHA and BHT as references; the test is repeated three times.

Measurements of the decrease in the absorbance of the DPPH radical caused by the presence of the extracts after 30 min allowed us to determine the antioxidant power of the different extracts. The inhibition power *I*(%) was expressed in the presence of different dilutions depending on the concentration after 30 min incubation at room temperature. *I*(%) is calculated using Equation (1):

$$I\ (\%) = \frac{(A_0 - A_i)}{A_0} \times 100 \qquad (1)$$

where I(%): percentage of inhibition, A_0 : absorbance of the DPPH solution in the absence of the reducing extract, A_i: absorbance of the DPPH solution in the presence of the reducing extract after 30 min incubation.

Bleaching test for β-carotene.

This method consists of measuring at 470 nm the discoloration of β-carotene resulting from its oxidation by the decomposition products of linoleic acid. The dispersion of linoleic acid and β-carotene in the aqueous phase was prepared with Tween 20. The oxidation of linoleic acid is catalyzed by heat (50°C). The addition of pure antioxidants or plant extracts induces a delay in the discoloration kinetics of β-carotene[13]. The study of antioxidant activity by the method of discoloration of β-carotene was conducted by the experimental protocol described by Ozsoy et al.[7]. To prepare the emulsion of β-carotene, 2 mg of the latter is dissolved in 10 mL chloroform, then 1 mL of this solution is mixed with 40 mg purified linoleic acid and 400 mg Tween 20. The chloroform is then evaporated under reduced pressure by a rotary evaporator and the residue obtained is taken up in 50 mL distilled water. Tubes containing 3 mL of this emulsion are prepared, to which 50 µL of prepared extracts or reference antioxidants (BHA) at different concentrations are added. The mixture is stirred well and the absorbance reading at 470 nm is immediately taken at t_0 against a blank containing the emulsion without β-carotene. The covered tubes are placed in a water bath set at 50°C and the absorbance reading is taken every 15 min during 120 min. A negative control is performed in parallel, comprising 3 mL of the emulsion of β-carotene and 50 µL absolute ethanol. The results obtained are expressed in terms of percentage inhibition of discoloration of β-carotene using Equation (2):

$$AA = \left(1 - \frac{A_0 - A_t}{A_{c0} - A_{ct}}\right) \times 100 \qquad (2)$$

where A_0 and Ac_0 are the absorbance values measured at zero times during the incubation for each fraction and control, respectively. A_t and A_{ct} were the absorbance values measured for each fraction and control, respectively, after incubation for 120 min. The results were expressed as IC_{50}.

Statistical analysis.

Experimental data were evaluated using an analysis of variance (ANOVA) and significant differences between the means of three replicates ($P>0.05$) were determined by Duncan's test, using the "Minitab17".

Results And Discussion

Sterol content.

A series of solutions were prepared using a cholesterol chloroform solution with a concentration of 1 mg/ml,. in order to draw a calibration curve of optical density-binding cholesterol as a function of concentration. The results presented in Table 1 clearly show that the total sterol contents of the oils in the studied samples of *N. sativa* L. seeds are significant (14.38 to 28.88 mg/g). These high levels may be explained by interference with other compounds that have chemical structures similar to the sterol structure such as methyl sterols and triterpene alcohols, vitamin D, β-carotene and other compounds that absorb at the assay wavelength. The analysis of all the results obtained clearly shows that the levels of sterolic compounds decreased ($p<0.05$) in the course of the roasting time (t), and the highest level (2.888 mg/g oil) is recorded at t = 5 min and the minimum value (1.438 mg/g oil) at t = 40 min.

Table 1. Level of sterols in the fixed oils of roasted *N. sativa* L. seeds.

Heat treatment time (*t*) at 180°C	Content (mg/g oil)
Untreated	2.8815 ± 0.21
5 min	2.888 ± 0.243
10 min	2.747± 0.26
15 min	2.75 ± 0.134
20 min	2.624 ± 0.318
30 min	2.395 ± 0.65
40 min	1.438 ± 0.41

Fatty Acid Composition.

Fatty acid analysis allows us to evaluate the stability and nutritional quality of the studied oils. A higher degree of oil establishment makes it more susceptible to oxidative degradation[14]. Fatty acid composition varies according to several factors, including variety, growing area, climate and seed maturity[15]. From the chromatograms of the fatty acid methyl esters (FAME) of oils, eleven fatty acids were identified (Table 2). Linoleic acid (18: 2) was found to be the primary fatty acid in the unroasted fixed *N. sativa* oil (58.854%). The contents of oleic acid (18: 1) and palmitic acid (16: 0) were 10.619 and 17.207% of the total fatty acids, respectively. Other fatty acids were found at relatively lower levels. For oils from different *N. sativa* seeds treated for different periods, the average levels were 10.148% oleic acid, 60.984% linoleic acid and 17.574% palmitic acid. From these results, it can be concluded that roasting did not change the fixed oils' fatty acid composition($p>0.05$) of the seeds of *N. sativa* L. Similar research was done by Karabulut *et al.*[16] on apricot kernels, they found that the fatty acid content does not change by roasting. The same result was confirmed by Juániz *et al.*[17] with green pepper. furthermore, Maheswary *et al.*[18] found that the fatty acid content has a significant dependence with the heat pre-treatment (roasting) in case of sunflower oils.

Table 2. Fatty acid composition in the fixed oils of treated and untreated *N. sativa* L. seeds.

	Heat treatment time at 180°C						
Fatty acid	**untreated**	**5 min**	**10 min**	**15 min**	**20 min**	**30 min**	**40 min**
C14:0	0.211±0.002	0.259±0.005	0.195±0.023	0.202±0.021	0.189±0.026	0.209±0.054	0.208±0.014
C16:0	17.207±1.123	17.574±0.001	16.988±0.004	16.988±0.031	16.818±0.002	17.575±0.129	16.913±0.065
C16:1	0.262±0.001	0.234±0.001	0.304±0.003	0.260±0.012	0.200±0.002	0.254±0.031	0.207±0.087
C18:0	4.329±0.025	3.934±0.021	3.935±0.014	3.913±0.002	3.888±0.004	4.034±0.009	3.910±0.006
C18:1	10.619±0.256	9.902±0.547	10.148±0.214	10.093±0.023	10.094±0.021	10.404±0.005	10.030±0.023
C18:2	58.854±1.564	60.156±1.147	60.984±0.875	61.152±0.022	60.701±0.002	60.014±0.013	60.064±0.783
C18:3	5.466±0.153	5.525±0.256	5.404±0.004±	5.414±0.001	5.533±0.005	5.322±0.235	5.677±0.002
C20:0	0.893±0.023	0.747±0.004	0.826±0.001	0.936±0.025	0.748±0.023	0.873±0.012	1.292±0.043

C20:1	0.378±0.001	0.356±0.001	0.271±0.003	0.407±0.002	0.545±0.043	0.381±0.003	0.509±0.033
C22:0	1.064±0.051	0.776±0.054	0.340±0.002	0.220±0.001	0.762±0.001	0.529±0.004	0.832±0.001
C22:1	0.716±0.003	0.537±0.025	0.607±0.005	0.551±0.003	0.612±0.068	0.404±0.001	0.358±0.016
Total SFA	23.704±1.231	21.510±0.261	20.924±0.001	20.902±0.023	20.708±0.651	21.611±0.456	22.116±0.023
Total IFA	76.295±1.002	75.584±0.026	76.537±0.003	76.660±0.156	76.329±0.236	75.741±0.123	75.772±0.021

Tocopherol analysis by High-performance liquid chromatography (HPLC).

The contents of the different tocopherols and tocotrienol in the oils prepared at different roasting residence times are gathered in Table 3. Three tocopherol homologues, namely α, δ- and γ-tocopherols, and one tocotrienol homologue, namely α –tocotrienol are detected by HPLC (Figure 1).

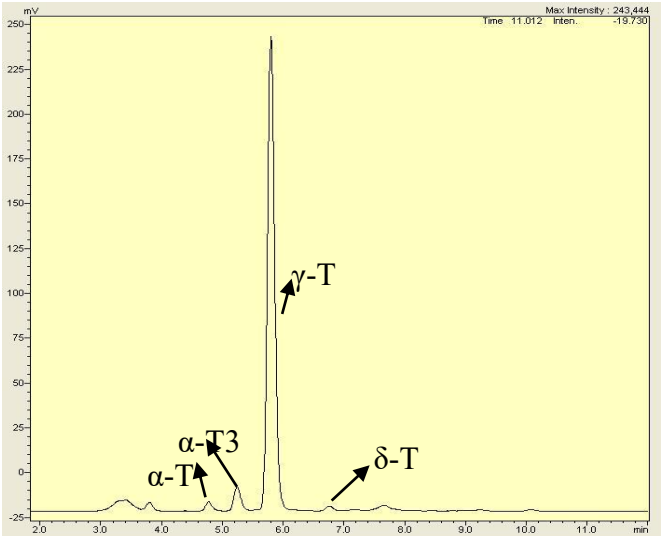


Figure 1. High-performance liquid chromatography (HPLC) chromatogram of the tocopherols in the fixed oils of the seeds of *N. sativa* L.

The gamma isomer was found to be the principal isomer of tocopherol, while the tocopherols α and δ were present in relatively small amounts and β-tocopherol was absent in the oil of the seeds of *N. sativa* L. The content of γ-tocopherol for the unroasted sample was measured at 1989 mg/kg oil (Table 3), and the content of γ-tocopherols in samples roasted for 5, 10, 15, 20, 30 and 40 min were quantified as 1384.1, 1073.9, 1479.1, 1848.4, 1760.7 and 1807.6 mg/kg oil, respectively.

Table 3. Content of individual tocopherols (mg/kg) in the fixed oils of treated *N. sativa* L. seeds.

Heat treatment time (*t*) at 180°C	Compounds			
	α-tocopherol	**α-tocotrienol**	**γ-Tocopherol**	**δ-Tocopherol**
untreated	64.1±0.4	127.6 ± 0.7	1989.0±31.6	23.3 ± 0.8
5 min	49.6 ± 2	77.9 ± 2.9	1384.1±55.4	17.9 ± 0.7
10 min	57.3 ± 2	52 ± 0.4	1073.9±58.7	18.9 ± 0.1
15 min	70.7±4.7	84.8 ± 4	1479.1±24.8	18.6 ± 1.4
20 min	58.3±0.1	113.7 ± 0.9	1848.4±44.4	19.8 ± 0.1
30 min	55.1±2.2	104 ± 4.9	1760.7±154	18.6 ± 0.5
40 min	63.7±2.2	99.2 ± 0.3	1807.6±61.4	18.7 ± 0.4

The content of γ-tocopherol in the oil of *N. sativa* L. after 5 min and 10 min of heat treatment displayed a remarkable decrease ($p<0.05$) after 15 min, an increase was observed to reach a maximum of 1848.4 mg/kg. After 20 min of roasting, this content begins to decrease even if in a slower way. Similar trends have been observed in α-tocopherol and α-tocotrienol. However, there were no differences in the content of δ-tocopherol. These results are in agreement with the findings mentioned by Durmaz et al.[7]. The reduction of tocopherols in oils prepared from seeds of *N. sativa* treated at high temperature could be explained by the thermal decomposition of tocopherols and the chemical reaction (esterification) of tocopherols with carboxylic acid fractions of amino acids of peptides and proteins at high roasting temperature[19]. In contrast, Gow-Chin Yen [20] reported that the level of tocopherol in heat-treated sesame oils was increased

Total phenolic content.

In most studies, the total phenolic content is measured to estimate the contribution of these substances to the antioxidant activity[21, 22]. The quantification of polyphenols in the various extracts of seeds of *N. sativa* L. was carried out using colorimetric methods (Folin-Ciocalte reagent and alumina trichloride). A calibration curve was established with gallic acid as the standard at various concentrations. The results were expressed in mg of gallic acid equivalent (GAE, as mg GAE/kg oil). The results obtained are grouped in Table 4.

Table 4. Total polyphenol content of the fixed oils of treated *N. sativa* L. seeds.

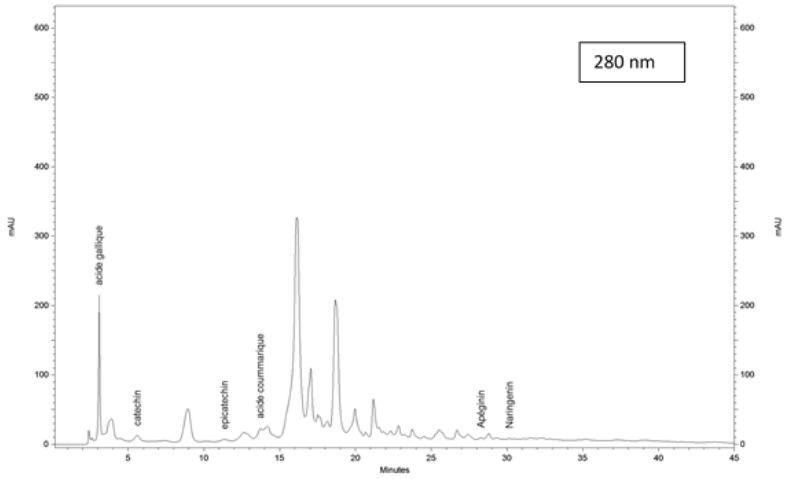
Heat treatment time at 180°C	Total polyphenol content (mg GAE/kg oil)
Untreated	8.00 ± 0.0001
5 (min)	6.40 ± 0.0005
10 (min)	7.60 ± 0.0016
15 (min)	12.1 ± 0.002
20 (min)	9.00 ± 0.0003

30 (min)	9.30 ± 0.0003
40 (min)	10.60 ± 0.0005

According to the synthesis of the set of results obtained during the quantification of the total phenols obtained for each extract, it can be seen that the contents of these compounds vary between 6.4 and 12.1 mg GAE/kg of oil (Table 4). The levels of the highest phenolic compounds were detected in samples treated for 15 min and 20 min($p<0.05$), while the lowest level was found in samples treated for 10 min (Table 4). In addition, we have followed the evolution of the polyphenol content as a function of the roasting time and we have recorded a gradual increase in the content between 5 min and 15 min($p<0.05$). Similar results were obtained in roasted cocoa liquor[23] the roasting considerably increases the total content of polyphenols. This increase is mainly related to the relatively polar compounds contained in the oil that have accumulated during roasting. Phenolic compounds would pass well into the oil phase if the oil was produced from roasted seeds. This is possibly due to the release of phenolic compounds[14] from the bound structures or chemical alteration of the phenolic compounds at higher temperatures[24]. When the heat treatment time exceeds 20 min, the polyphenol content starts to decrease slightly as a content of 9.3 mg GAE/kg oil was recorded between 30 and 40 min.

Individual phenolic identification by HPLC.

HPLC coupled with a photodiode array detector was adopted to separate and quantify phenolic compounds. These compounds were identified based on their retention times and the spectral characteristics of their peaks relative to those of the standards, as well as by spiking the sample with standards. The chromatographic profile of the phenolic extracts of the samples studied revealed the presence of the phenolic acids identified at 280 nm (figure 2). There were three known phenolic acids in all extracts: gallic acid, vanillic acid, and coumarin acid (and unknown compounds). HPLC analysis of flavonoids revealed the presence of four compounds at 360 nm (figure 2): rutin, apigenin, kaempferol and epicatichine. The most abundant compounds in all samples of *N. sativa* L. seeds were gallic acid for phenolic acids and rutin for flavonoids. Phenolic acids and flavonoids identified in *N. sativa* L. seeds by other researchers[25] were not detected in our samples.



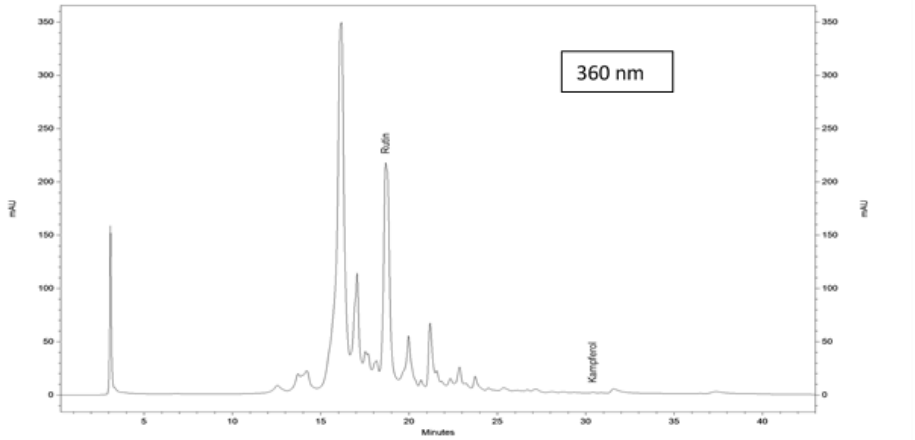


Figure 2: HPLC chromatograms of the phenolic compounds of the fixed oil of seeds of Nigella sativa L. roasted for 30 min at 280 nm and 360 nm

This is probably due to varietal differences such as chlorogenic acid, p-dihydroxybenzoic acid, ferulic acid, trans-2hydroxycinnamic acid,(-)-Epicatechine,(+)-Catechine and isorhamnetin[25]. The results presented in Table 5 proved that differences in the phenolic composition of the fixed oils of treated *N. sativa* L. seeds were significantly more quantitative than qualitative. With the exception of epicatechine that is detected only in the fixed oils of seeds treated for 15, 20, 30 and 40 min.

Table 5. Content (µg/g oil) of phenolic acids and flavonoids in the fixed oils of roasted *N. sativa* L. seeds.

compound	Heat treatment time (*t*) at 180°C						
	Untreated	5 min	10 min	15 min	20 min	30 min	40 min
Gallic Acid	37.12 ± 0.13	13.01± 0.13	12.79 ±0.05	1110.86 ±21.00	982.46 ±8.14	236.17±11.06	212.68 ±7.85
Catéchin	ND*	ND	ND	ND	ND	ND	ND
Vanillic acid	ND	ND	ND	1030.37 ±2 .14	920.91 ±15.08	9.02 ±0.08	6.39 ±0.11
Epicatechin	ND	ND	ND	735.61 ±12.18	1700.48 ±11.56	275.79 ± 2.14	80.76 ± 5.2
Coumaric acid	ND	ND	ND	221.73 ± 0.574	212.86 ±0.50	185.09 ± 5.87	65.16 ± 2.01
Apigenin	45.38 ± 2.53	27.80±2.30	23.61±0.67	129.80 ±8.52	949.45 ±17.8	82.58 ±4.38	13.34 ±0.04
Naringenin	ND	ND	ND	ND	ND	ND	ND
Rutin	118.34±0.42	8.87±1.44	8.85±0.27	4297.11±15.6	2096.11 ±18.06	2650.84±22.14	370.18 ±1.25
Quercetin	ND	ND	ND	ND	ND	ND	ND
Kaempferol	5.42 ± 0.21	0.79±0.01	0.81±0.03	28.86 ± 2.10	5.85 ± 0.05	8.04 ± 0.5	ND

*ND: not detected

We also followed the evolution of abundant compounds. For gallic acid, the value recorded for fixed oils extracted from seeds treated for 5 min is 13.12 (µg/g of oil) ($p<0.05$). For seeds treated for 10 min, the

recorded value is 12.79 (µg/ g) oil), we can affirm that there is no change. After 15 min of roasting, A significant rise in gallic acid content of about 1110.86 (µg/g of oil)($p<0.05$) was observed.. This increase is caused by the deterioration of the hydrolyzable tannins derived from gallic acid[19]. After 20 min of roasting, the gallic acid content begins to decrease, to reach 982.46 (µg/g of oil) after 40 min($p<0.05$) of heat treatment. Similar tendencies were observed for rutin (Table 5), and a significant increase was recorded at 15 min of roasting (4297.11 µg/g of oil). This content decreased from 20 min of roasting to reach 2096.11 (µg/g of oil) after 40 min. For minor compounds (like kaempferol, apigenin and vanillic acid), we noticed the same trends as the other major compounds. The content of these compounds decreased significantly to reach a minimal value. The important point in this analysis is the appearance of epicatichine in the oils of seeds treated for 15, 20, 30 and 40 min and it was absent in the oils of seeds untreated and treated for 5 and 10 min. The heat treatment is known to cause the alteration of the chemical structure of certain molecules, including tannins[19] that are generally oligomers and polymers of catechin and epicatechin whose alteration liberates the epicatichins in the fixed oils causing the increase of its content[26]. These results indicate that during the heat treatment process, there will be a remarkable increase in all the phenolic compounds detected and that the best treatment time is 15 min. After this time, all the compounds decreased due to the effect of temperature. These results agree well with the literature[27].

Evaluation of the antioxidant activity of the fixed oils.

The antioxidant activity of the different oils of the seeds of *N. sativa* L treated for several moment and of the standard antioxidants (hydroxytoluene-butyl, BHT, and hydroxyanisolebutyl alcohol, BHA) was evaluated with a spectrophotometer UV-Vis, IR, etc. The results obtained showed that the extracts have a very high antioxidant power compared to other oils such as Apricot Kernel Oil[7] and Argon Oil[28]. This activity is comparable to that of the reference antioxidants BHT (922.0391 mL/mg) and BHA (787.6289 mL/mg). However, standard antioxidants are three times more active than our examined extracts.

Table 6. *IC*50 values of the different oils of the treated seeds during different times in the DPPH test and bleaching test of β-carotene.

Heat treatment time at 180°C	DPPH test IC_{50} (mg/mL)	Bleaching test of β-carotene IC_{50} (mg/mL)
Untreated	0.0197 ± 0.0003	0.0299 ± 0.0002
5	0.0066±0.0001	0.0027 ± 0.0001
10	0.0039±0.0004	0.0021 ± 0.0004
15	0.0032±0.0003	0.0015 ± 0.0003
20	0.004±0.0001	0.0017 ± 0.0003
30	0.0039±0.0002	0.0017 ± 0.0001
40	0.0057±0.0003	0.0019 ± 0.0002
BHT	0.0011 ± 0.0001	0.0005 ± 0.00001
BHA	0.0013 ± 0.0003	0.0073 ± 0.0004

From the results obtained during this study, it can be noted that the fixed oils obtained from untreated *N. sativa* L. seeds showed anti-free radical properties ranging from 0.0032 to 0.0197 mg/mL in the DPPH test and from 0.0015 to 0.0299 mg/mL in the β-carotene bleaching test. The heat treatment resulted in a clear increase in antioxidant activity as measured by DPPH and bleaching β-carotene tests (Table 6).

The antioxidant activity gradually increased during roasting process, reaching an apparent maximum in less than 15 min.

A slight decrease in the antioxidant capacity of the samples was observed after a 20 min roasting time($p<0.05$). In contrast, the oil from the grains treated for 5 min had a low anti-free radical status. That result is coherent with.the literature as most researches showed that the ideal roasting time is between 15 and 20 min[7, 24, 29, 30]. However, the heat treatment of the seeds significantly increased the levels of gallic acid, vanillic acid, epicatechin, coumaric acid, Apigenin, Naringenin, Rutin, Quercetin and Kaempferol, These results suggest that these phenols may be the main bioactive compounds responsible for this plant's antioxidant activity [31].

Conclusion

The fixed oils of *N. sativa* L. seeds showed an important content of bioactive compounds (tocopherols and phenolic compounds) as well as a considerable antioxidant capacity, suggesting that these fixed oils could be a potential source of antioxidants. The effectt of the roasting process on the antioxidant activity and the composition of bioactive compounds in the fixed oils of *N. sativa* L. seeds has been investigated. Oils obtained from roasted beans lay (15 to 20 min) with a high polyphenol content and antioxidant activity. However, compared to an unroasted sample, shorter (5-10 min) or longer (30 - 40 min) roasting periods caused a counter-effect on antioxidant activity and polyphenol content. On the other hand, the level of tocopherols was lowered by roasting.

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References

1. Hussain, D. and M.M. Hussain, *Nigella sativa (black seed) is an effective herbal remedy for every disease except death-a Prophetic statement which modern scientists confirm unanimously: a review.* Adv Med Plant Res, 2016. **4**(2): p. 27-57.
2. Ghannadi, A., V. Hajhashemi, and H. Jafarabadi, *An investigation of the analgesic and anti-inflammatory effects of Nigella sativa seed polyphenols.* Journal of medicinal food, 2005. **8**(4): p. 488-493.
3. Jean-Paul, P.B., *La Nigelle, une panacée peu connue en Occident.* Université de Bourgogne, 1987.
4. Chang, S.K., et al., *Nuts and their co-products: The impact of processing (roasting) on phenolics, bioavailability, and health benefits–A comprehensive review.* Journal of functional foods, 2016. **26**: p. 88-122.

5. Wani, I.A., et al., *Physico-chemical, rheological and antioxidant properties of sweet chestnut (Castanea sativa Mill.) as affected by pan and microwave roasting.* Journal of advanced research, 2017. **8**(4): p. 399-405.
6. Jennings, B.H. and C.C. Akoh, *Enzymatic modification of triacylglycerols of high eicosapentaenoic and docosahexaenoic acids content to produce structured lipids.* Journal of the American Oil Chemists' Society, 1999. **76**(10): p. 1133-1137.
7. Durmaz, G., et al., *Roasting-related changes in oxidative stability and antioxidant capacity of apricot kernel oil.* Journal of the American Oil Chemists' Society, 2010. **87**(4): p. 401-409.
8. Laghouiter, O.K., et al., *Chemical characterization and in vitro antioxidant capacity of nine Algerian date palm cultivars (Phoenix dactylifera L.) seed oil.* Mediterranean Journal of Nutrition and Metabolism, 2018. **11**(2): p. 103-117.
9. Karabulut, I., et al., *Effects of the industrial refining process on some properties of hazelnut oil.* European journal of lipid science and technology, 2005. **107**(7-8): p. 476-480.
10. Singleton, V.L., R. Orthofer, and R.M. Lamuela-Raventós, *[14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent*, in *Methods in enzymology*1999, Elsevier. p. 152-178.
11. Ben Saad, A., et al., *Phytochemical, antioxidant and protective effect of cactus cladodes extract against lithium-induced liver injury in rats.* Pharmaceutical biology, 2017. **55**(1): p. 516-525.
12. Espín, J.C., C. Soler-Rivas, and H.J. Wichers, *Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2, 2-diphenyl-1-picrylhydrazyl radical.* Journal of Agricultural and Food chemistry, 2000. **48**(3): p. 648-656.
13. Cirillo, G., et al., *Starch-quercetin conjugate by radical grafting: synthesis and biological characterization.* Pharmaceutical development and technology, 2012. **17**(4): p. 466-476.
14. Sherwin, E., *Oxidation and antioxidants in fat and oil processing.* Journal of the American Oil Chemists' Society, 1978. **55**(11): p. 809-814.
15. Worden, A.Z., et al., *Green evolution and dynamic adaptations revealed by genomes of the marine picoeukaryotes Micromonas.* Science, 2009. **324**(5924): p. 268-272.
16. Karabulut, I., G. Durmaz, and A.A. Hayaloglu, *Fatty acid selectivity of lipases during acidolysis reaction between oleic acid and monoacid triacylglycerols.* Journal of Agricultural and Food chemistry, 2009. **57**(21): p. 10466-10470.
17. Juániz, I., et al., *Influence of heat treatment on antioxidant capacity and (poly) phenolic compounds of selected vegetables.* Food chemistry, 2016. **197**: p. 466-473.
18. Veldsink, J.W., et al., *Heat pretreatment of oilseeds: effect on oil quality.* Lipid/Fett, 1999. **101**(7): p. 244-248.
19. Lemos, M.R.B., et al., *The effect of roasting on the phenolic compounds and antioxidant potential of baru nuts [Dipteryx alata Vog.].* Food Research International, 2012. **48**(2): p. 592-597.
20. Yen, G.-C. and S.-L. Shyu, *Oxidative stability of sesame oil prepared from sesame seed with different roasting temperatures.* Food chemistry, 1989. **31**(3): p. 215-224.

21. Arnao, M.B., A. Cano, and M. Acosta, *Methods to measure the antioxidant activity in plant material. A comparative discussion.* Free Radical Research, 1999. **31**(sup1): p. 89-96.
22. Hwang, I., H.-C. Chen, and J. Sheen, *Two-component signal transduction pathways in Arabidopsis.* Plant Physiology, 2002. **129**(2): p. 500-515.
23. Jinap, S., B. Jamilah, and S. Nazamid, *Sensory properties of cocoa liquor as affected by polyphenol concentration and duration of roasting.* Food Quality and preference, 2004. **15**(5): p. 403-409.
24. Durmaz, G. and V. Gökmen, *Changes in oxidative stability, antioxidant capacity and phytochemical composition of Pistacia terebinthus oil with roasting.* Food chemistry, 2011. **128**(2): p. 410-414.
25. Bourgou, S., et al., *Phenolic composition and biological activities of Tunisian Nigella sativa L. shoots and roots.* Comptes Rendus Biologies, 2008. **331**(1): p. 48-55.
26. Kokaly, R.F. and A.K. Skidmore, *Plant phenolics and absorption features in vegetation reflectance spectra near 1.66 μm.* International Journal of Applied Earth Observation and Geoinformation, 2015. **43**: p. 55-83.
27. Rodrigues, A., et al., *Effect of curing and cooking on flavonols and anthocyanins in traditional varieties of onion bulbs.* Food Research International, 2009. **42**(9): p. 1331-1336.
28. Rahmani, M., *Composition chimique de l'huile d'argane «vierge».* Cahiers agricultures, 2005. **14**(5): p. 461-465 (1).
29. Chu, Y.-H., *Effects of soybean pretreatments on crude oil quality.* Journal of the American Oil Chemists' Society, 1995. **72**(2): p. 177-181.
30. Ghedadba, N., et al., *Évaluation de l'activité antioxydante et antimicrobienne des feuilles et des sommités fleuries de Marrubium vulgare L.* Phytothérapie, 2014. **12**(1): p. 15-24.
31. Jung, M., et al., *Pyrazine contents and oxidative stabilities of roasted soybean oils.* Food chemistry, 1997. **60**(1): p. 95-102.