

Article

Effects of Stripping Essential Oil from *Nigella sativa* L. Oil on Its Quality Characteristics

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Abstract

Black cumin (*Nigella sativa* L.) oil is one of the most important vegetable oils with food, therapeutic and medicinal uses; however, it has high acid and peroxide values even in fresh oil extracted by cold pressing. One of the main factors that can cause high peroxide value (PV) and acid value (AV) in the extracted black cumin oil (BO) is the presence of essential oils (EO), which are highly sensitive to oxidation. In this study, separation by steam was used to strip EO from BO, and the obtained oil quality was evaluated during storage in room conditions for 90 days. The AV and PV in BO without EO were lower and showed a lower increase compared to BO with EO during storage. Chlorophyll, carotenoid, thymoquinone, phytosterols and tocopherols were lower in samples without EO than those with EO. Fatty acid composition was similar in both types of oils, but linoleic acid (18:2) was decreased at a higher rate in the oil with EO during storage. Therefore, the obtained results show that steam stripping of EO from BO can be suggested to obtain oil with lower PV and AV and with a relatively longer shelf life.

Keywords: *Nigella sativa* L.; essential oil; oxidation; shelf life; bioactive components

1. Introduction

Black cumin (*Nigella sativa* L.) seed is a valuable source of bioactive components. Black cumin seed and its oil have been used for a long time. This seed is native to Southwest Asia, but it is currently cultivated in different countries such as India, Pakistan, Iran, Turkey, Syria, and Saudi Arabia. Black cumin has a high content of oil (20–35%), which is widely used in many foods, cosmetic and pharmaceutical products [1,2].

Unlike other oilseeds, black cumin has a high content of essential oils (EO). These essential oils are present in the black cumin seed and also in the oil extracted from the seeds. They contain different compounds, mainly including hydrocarbons, monoterpenes, and sesquiterpenes in the EO. The main component of EO is *p*-cymene, followed by thymoquinone, carvacrol and camphene [3].

EOs can act as antioxidative components, but these components are sensitive to oxidation and can also be oxidized very fast. For example, oxidation can take place in substituting methyl and isopropyl groups of the *p*-cymene molecule (one of the EO components) and generate *p*-cymene hydroperoxide [4]. It has been reported that *p*-cymene



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decreased during storage, and its content was reduced up to 43% during storage for 6 months, which can affect the black cumin oil (BO) peroxide value (PV) [5].

BO can be extracted by pressing or using a solvent. Pressing can also be done by hot or cold pressing. Oil extracted via solvent or hot pressing has to be refined; therefore, cold pressing is preferred as there is no need for refining steps if the extracted oil is of high quality. There are many reports indicating that even fresh BO has high PV regardless of the extraction method. One reason can be the EO, which can be oxidized, and during PV determination, the general iodimetry methods can be considered as peroxides of oil samples. These phenomena are seen in herbal extracts with high EO, which have high PV as well. In addition, oxygenated components in the EO can affect the PV of oil samples [6].

Despite the many therapeutic and nutritional properties of BO, this oil has very low oxidative stability and has a high peroxide and acid value (AV). The PV of this oil is significantly higher than that of other oils extracted from oil seeds [7]. Therefore, it requires the use of different methods in order to control the increase in the PV from the moment of extraction. Several approaches have been carried out to reduce the oxidation of BO, such as seed pretreatments, blending with other edible oils and medicinal plants, and using antioxidants [7], but they have not been very successful in controlling the PV of BO.

One of the modern methods for increasing the shelf life of oils is the use of hot water vapor and a vacuum system to separate essential oils from the oil. This method uses high temperatures and a very short time for extracting the essential oils, which reduces the effect of EO degradation and decomposition effects on the oil and increases the shelf life of the oil. Therefore, the aim of this research was to separate and strip the EO from the BO by steam to evaluate its effects on peroxide value, acid value, pigments (chlorophyll and carotenoid) and bioactive components such as thymoquinones, phytosterols and tocopherols. In addition, the effects of EO stripping on the oxidative stability and shelf life of the BO were evaluated during storage.

2. Materials and Method

2.1. Materials

2.1.1. Seed Sample

Black cumin seeds were purchased from the local market (Tabriz, Iran).

2.1.2. Chemicals

KOH, acetic acid, chloroform, iodine, sodium thiosulfate, cyclohexane, gallic acid equivalents, Folin–Ciocalteu reagent, sodium carbonate, *n*-heptane, tert-butyl methyl ether, tetrahydrofuran, and methanol were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2. Cold-Press Oil Extraction Method

Black cumin seeds were cleaned, and then the seeds were poured into the tank of the cold press machine (Screw Press Model 85 mm, Kern Kraft, Hamburg, Germany) for oil extraction. The temperature of the extracted oil was monitored to maintain it below 40 °C during the oil extraction. After oil extraction, oil samples were divided into two portions. One portion was kept in a dark glass bottle at room temperature to evaluate its quality changes and PV during storage for 90 days. The other portion was used to separate its EO as described below.

2.3. Separation of Essential Oil

BO (200 g) was poured into a glass balloon, and pressurized steam was injected into the oil. After a few seconds, the vapor containing the essential oils of BO was separated

and collected by the condenser. To reduce the oil temperature, the balloon was placed in a container full of circulating cold water, and the entire system was vacuumed using a vacuum pump. A thermometer installed on top of the glass balloon was used to control the oil temperature (Figure 1). After stripping the EO, the oil sample was kept in a tightly sealed, dark glass bottle with minimal head space at room temperature ($25 \pm 2^\circ\text{C}$) in a dry and dark place to evaluate its quality changes during storage for 90 days. The samples were protected from excessive light and temperature fluctuations during sampling, and analyses were performed within a short timeframe after sample preparation to minimize oxidative degradation.

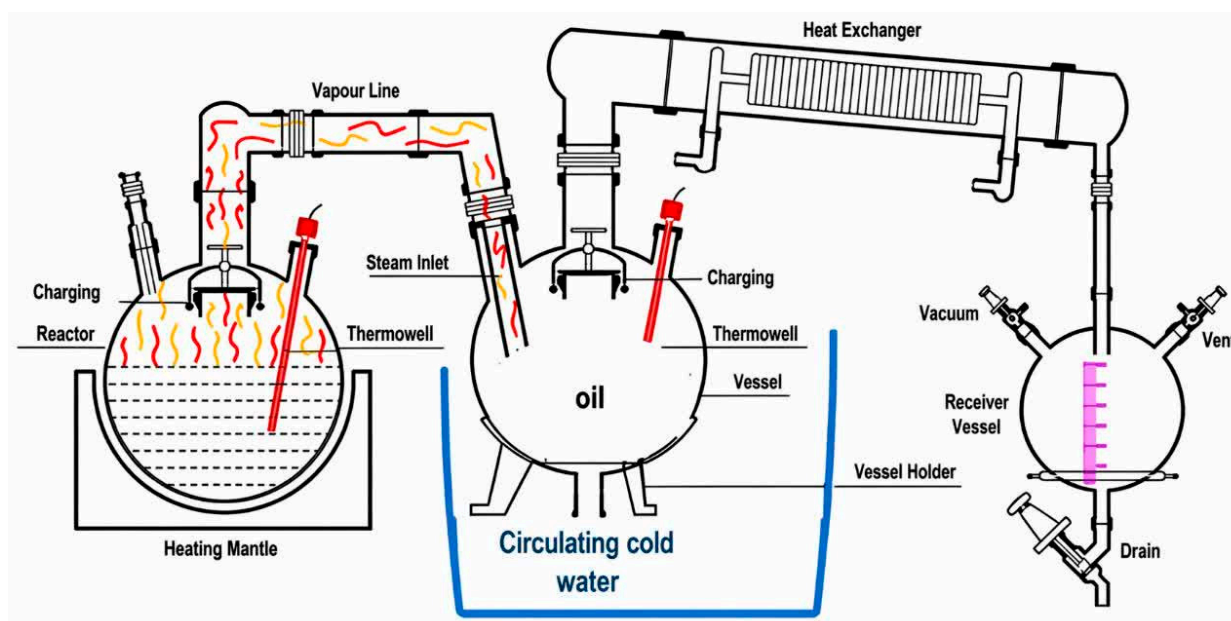


Figure 1. Separation system of essential oil from *Nigella sativa* L. seed oil.

2.4. Acid Value

The AV (mg KOH/g of oil) was determined via titration using the official method (Cd 5a–40) described by the American Oil Chemists' Society (1997) [8].

2.5. Peroxide Value

The PV (meq O_2 /kg of oil) was determined by reacting the oil dissolved in an acetic acid and chloroform mixture with iodine and starch solutions, followed by titration with sodium thiosulfate using an official method (Cd 8–53) described by the American Oil Chemists' Society (1997) [8].

2.6. Chlorophyll and Carotenoid Contents

Chlorophylls and carotenoids of BO were determined by dissolving the oil sample (7.5 g) in cyclohexane (25 mL) by measuring the absorbance at 670 nm and 470 nm using a spectrophotometer (UNICO, Phoenix Equipment, Inc, Rochester, NY, USA), respectively, according to the method described by [9].

2.7. Thymoquinone Content

Quantification of thymoquinone amount in oil samples was carried out using the High-performance liquid chromatography (HPLC) (Agilent 1200, Palo Alto, CA, USA) according to the method described by [10].

2.8. Rancimat Analysis

Rancimat Instrument (Metrohm 743; Metrohm Co., Herisau, Switzerland) was used to determine the oxidative stability of BO samples according to the official method (Cd 12b–92) described by the American Oil Chemists' Society (1997) [8].

2.9. Total Polyphenols Content

The total phenolic compounds (TPCs) of the BO were determined as Gallic Acid Equivalents (GAEs) by a spectrophotometer (UNICO, UK) at a wavelength of 760 nm after reaction of the oil sample with Folin–Ciocalteu reagent in the presence of sodium carbonate according to the previously published method [11].

2.10. Phytosterols Composition

Phytosterols of oil samples were determined following the ISO-12228 method (1999) [12]. For this purpose, gas chromatography (GC) (Agilent 7890 B, Agilent, Santa Clara, CA, USA) with a capillary column of HP-5 (30 m length $\times$ 0.32 mm i.d. $\times$ 0.25 μ m film thickness) was used for analysis. Helium with a flow rate of 2 mL/min was used as a carrier gas. The injector and flame ionization detector temperatures were set to 280 and 290 °C, respectively. The oven temperature was also programmed at 260 °C for 50 min.

2.11. Tocopherols Content

The tocopherol composition and content of oil samples were determined by HPLC using the method described by [13]. For this purpose, 10 μ L of the oil sample (10 mg) dissolved in *n*-heptane (1 mL) was injected into the HPLC coupled with the column of LiChroCART (250-4 packed with LiChrosphere 100 NH₂) (Merck KGaA, Darmstadt, Germany) and a fluorescence detector (DETECTOR Engineering & Technology, Inc., Walnut Creek, CA, USA). The mobile phase also involved a mixture of *n*-heptane:tert-butyl methyl ether:tetrahydro-furan:methanol (79:20:0.98:0.02, *v:v:v:v*), which was used at a flow rate of 1.0 mL/min. Different tocopherol isomers were used for the identification of tocopherols, and an external standard method was also used to determine the concentration of tocopherols.

2.12. Fatty Acid Composition

Fatty acid methyl esters (FAMES) of oil samples were prepared via transesterification for fatty acid analysis according to the previously published method [13]. Then, FAMES were analyzed by gas chromatography (GC, Agilent Technologies, Santa Clara, CA, USA) using a capillary column BPX70 (length of 50 m, inner diameter of 0.25 mm and particle size of the stationary phase of 25 micrometers) and helium as the carrier gas. The oven temperature for analysis was 158 °C for 5 min and then raised to 230 °C at a rate of 2 °C/min and remained at 230 °C for 5 min [13]. A flame ionization detector was used at a temperature of 250 °C to detect FAMES. Retention times of fatty acid standards were used to identify the FAME composition of oil samples. The area under the peaks was used to determine fatty acid percentages.

2.13. Statistical Analysis

All analyses were carried out in three replications, and data were presented as the mean $\pm$ standard deviation (SD). The obtained data were analyzed by the two-way analysis of variance (ANOVA) considering oil type (with essential oil vs. without essential oil) and storage time as fixed factors using SPSS statistical software (v22 software, Chicago, IL, USA). Also, Duncan's post hoc test was employed to determine the significant differences in means at $p < 0.05$.

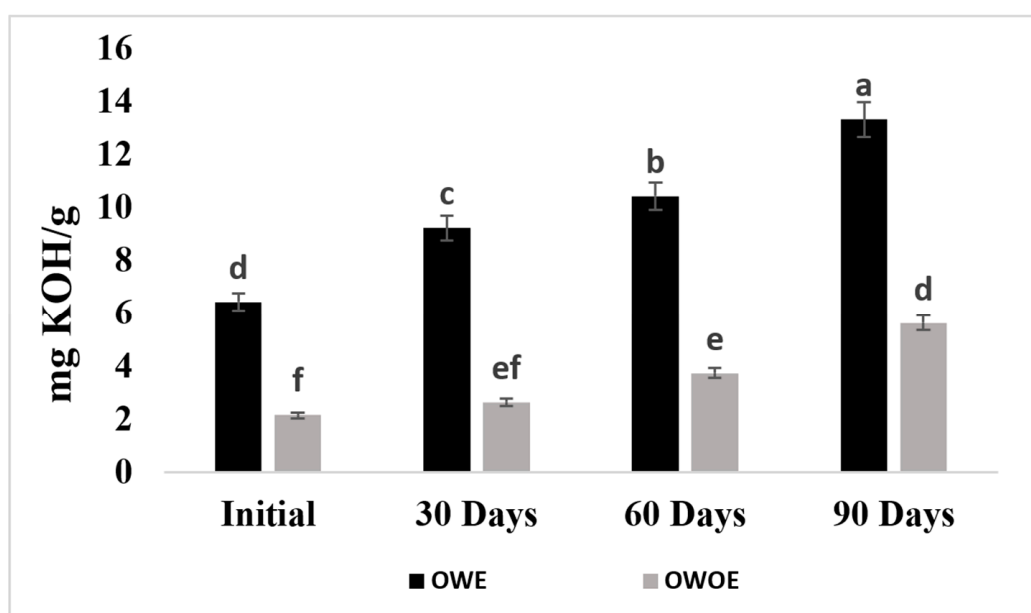
3. Results and Discussion

3.1. Acid Value (AV)

One of the important factors in checking the quality of edible oils is AV. A high AV indicates the hydrolysis of triacylglycerol and the production of free fatty acids. A high level of free fatty acids in the oil can reduce the smoke point and reduce the stability of the oil against oxidation. Lipase enzyme activity, oil storage in inappropriate conditions, and oil heating in the presence of moisture are factors influencing the AV and its changes [14].

Stripping EO could decrease the AV of BO, which can be related to the separation of FFA, as these compounds are volatile at high temperatures and low pressure applied during steam stripping. The AV of BO with EO was higher on processing day (day 1) and showed a significant increase ($p < 0.05$) compared with that of BO without EO during storage as well (Figure 2). This action may indicate that extracting EO from oil reduces the hydrolysis and formation of FFA during storage, which affects the AV. The lowest amount of AV (2.1%) was obtained for the oil without EO on day 1, and the highest amount (13.0%) was obtained on day 90 for oil with EO (Figure 2).

It has been reported that the increase in AV during storage can be attributed to the increase in the formation of free fatty acids from the hydrolysis of triacylglycerol in black seed oil [15]. The results of previous studies also indicated an increase in the amount of AV during 90 days of storage [16]. The obtained results were also in accordance with the fact that the AV increased during the storage of BO [14,17,18].



Source of Variation	df	MS	F	p-Value
Storage time	3	29.84	114.21 **	0.0004
Essential oil separation	1	245.44	939.5 **	0.0001
Essential oil separation × storage time	3	3.524	13.49 **	0.0009
Error	16	0.261		
CV (%)			7.66	

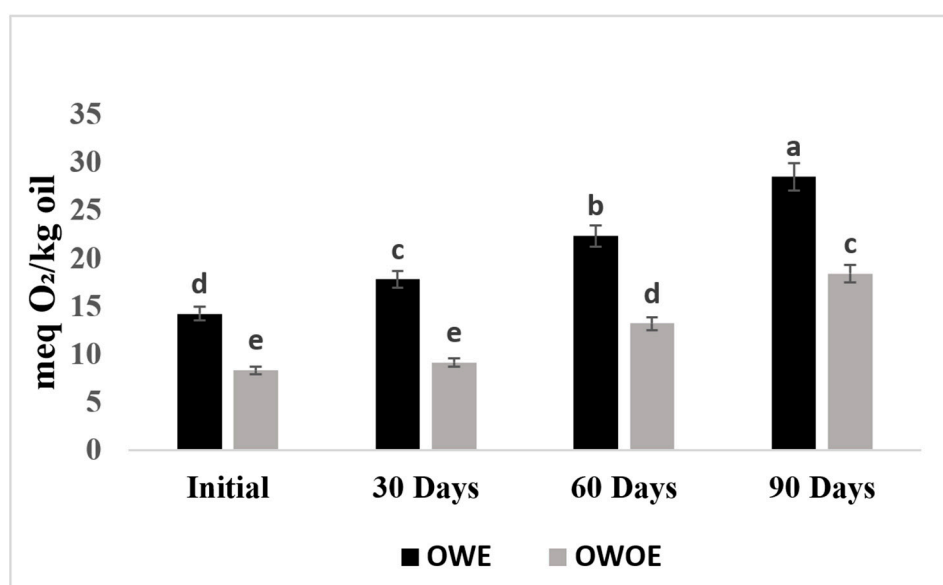
Figure 2. The interaction effect of storage time × essential oil separation treatment on the acid value of *Nigella sativa* oil. The means with different letters (a–f) are significantly different at $p \leq 0.05$. OWE: oil with essential oil, OWOE: oil without essential oil. (**: significant at $p \leq 0.01$).

3.2. Peroxide Value

The maximum PV for cold-pressed vegetable oils established by Codex Alimentarius is 15 (meq O₂/kg oil) [19]. The oil with high stability against oxidation has a low PV and shows a lower increase during processing, storage and heating [20,21]. Despite the many therapeutic and nutritional properties of black seed oil, this oil has low stability and high PV. The PV of this oil is significantly different from that of other oils extracted from oil seeds. Usually, this oil has a higher PV than other vegetable oils from the moment of extraction [2,4,7]. The increasing process of PV can be caused by more oxygen absorption by unsaturated fatty acids and oxidation [22].

As EO is very sensitive to oxidation, the oil with EO had significantly ($p < 0.05$) higher PV compared with the oil without EO on the extraction day and also during the storage period (Figure 3). The obtained results showed that the PV of oil samples was increased during storage; however, the PV of BO with EO showed a significantly higher increase ($p < 0.05$) than that of BO without EO, as some components in EO can be oxidized during storage. These results may indicate that the separation of EO from oil can reduce the EO oxidation products, which can affect the PV of oil samples during storage (Figure 3). These results were in accordance with the fact that the PV was increased during the storage of BO for 4 months [18,23].

There are many reports showing that PV of BO was increased during storage [14,17–19]. The obtained results in the present research are consistent with previous reports on the increase in PV of oil extracted from pretreated BS by microwave during storage [23].

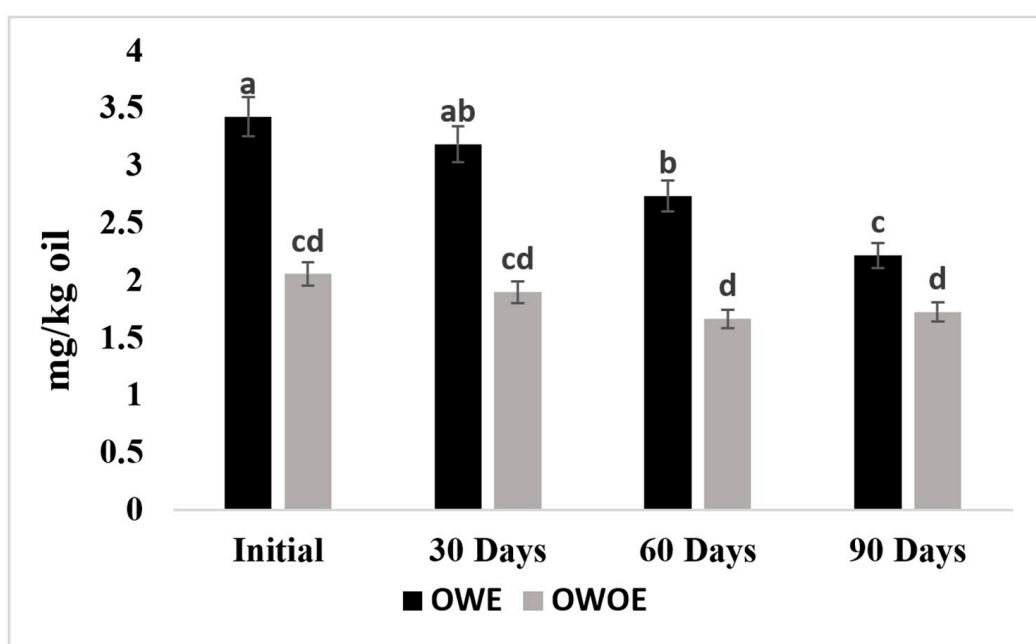


Source of Variation	df	MS	F	p-Value
Storage time	3	196.83	61.8 **	0.0006
Essential oil separation	1	462	145.06 **	0.0002
Essential oil separation × storage time	3	4.468	1.40 ns	0.278
Error	16	3.185		
CV (%)			11.33	

Figure 3. The interaction effect of storage time × essential oil separation treatment on peroxide value of *Nigella sativa* oil. The means with different letters (a–e) are significantly different at $p \leq 0.05$. OWE: oil with essential oil, OWOE: oil without essential oil. (ns and **: non-significant and significant at $p \leq 0.01$, respectively).

3.3. Chlorophyll and Carotenoid Contents

BO is rich in natural pigments such as carotenoids and chlorophylls. Chlorophyll in edible oils in the presence of light causes photooxidation and reduces the stability of the oil due to oxidation. Chlorophylls can act as a sensitizer in the presence of light and facilitate photooxidation. As a result of oxidation, PV of the oil can increase and finally may cause an unpleasant taste in the oil [20,24,25]. Stripping of EO could significantly decrease ($p < 0.05$) the chlorophyll content of striped oil (from 3.4 mg/kg oil in fresh oil to 2.0 mg/kg oil in stripped oil). Storage also had a significant effect on the chlorophyll content, as it decreased during the 90 days of storage in the oil sample with EO, which was associated with degradation reactions (Figure 4) [25]. Also, the obtained results showed that the chlorophyll content reduction in BO without EO was not affected significantly by storage (Figure 4). Similar results of the reduction in chlorophyll content of BO during storage have been reported [16].

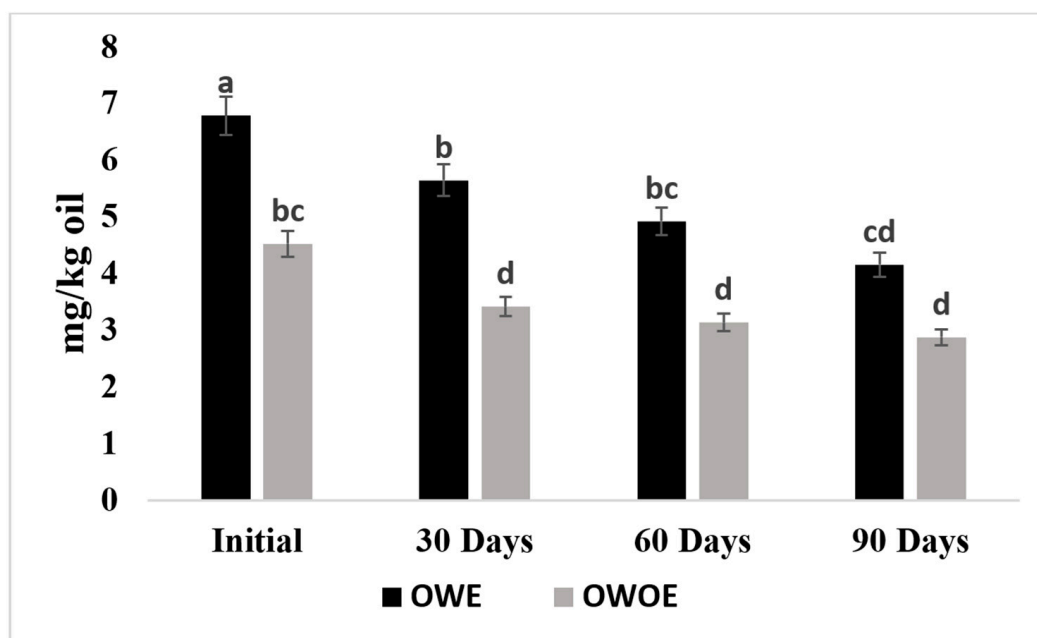


Source of Variation	df	MS	F	p-Value
Storage time	3	0.62	9.88 **	0.0001
Essential oil separation	1	6.62	104.63 **	0.0001
Essential oil separation × storage time	3	0.24	3.87 *	0.0294
Error	16	0.06		
CV (%)			10.81	

Figure 4. The interaction effect of storage time × essential oil separation treatment on chlorophyll content of *Nigella sativa* oil. The means with different letters (a–d) are significantly different at $p \leq 0.05$. OWE: oil with essential oil, OWOE: oil without essential oil. (* and **: significant at $p \leq 0.05$ and $p \leq 0.01$, respectively).

Carotenoids affect oil color as well as oil stability against oxidation and nutritional properties of vegetable oils [26]. The highest amount of carotenoid in oil with EO was on day 1 (6.7 mg/kg oil), and the lowest amount was in oil without EO on day 90 (2.8 mg/kg oil). EO separation could decrease the carotenoid content on the processing day (day 1) as these pigments are sensitive to heat, and processing at high temperatures can cause thermal bleaching and reduce the carotenoid content (Figure 5). Storage of oil samples could also

cause a significant reduction ($p < 0.05$) in carotenoid content of BO with EO; however, this reduction was not significant in BO without EO after 30 days of storage (Figure 5). The obtained results are consistent with the data that have been reported regarding the reduction in carotenoids during 90 days of storage of BO at room temperature [16]. Carotenoids can be reduced during heat treatment, such as steam separating during EO separation, and also during storage via decomposition or oxidation [14].



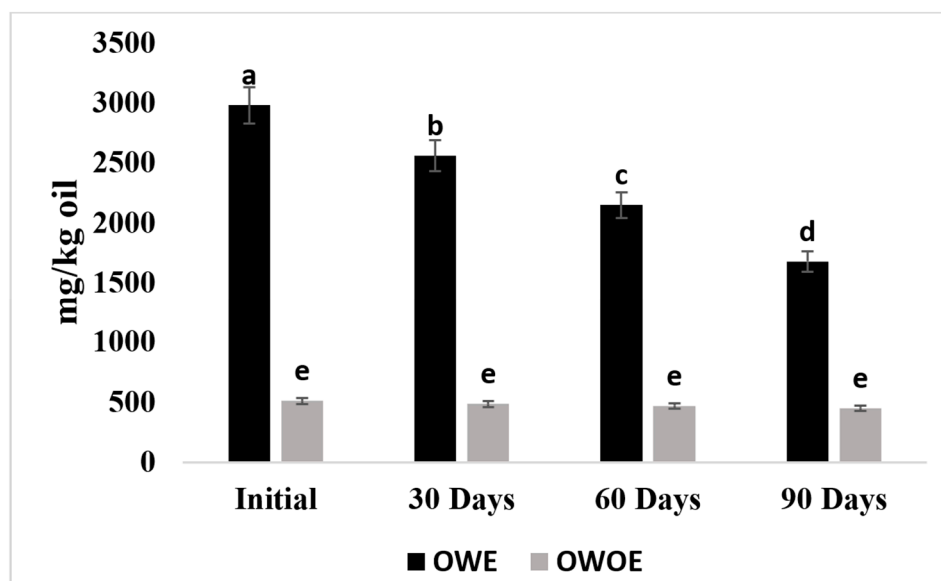
Source of Variation	df	MS	F	p-Value
Storage time	3	5.72	14.54 **	0.0005
Essential oil separation	1	19.42	49.39 **	0.0001
Essential oil separation × storage time	3	0.34	0.86 ns	0.4808
Error	16	0.39		
CV (%)			14.42	

Figure 5. The interaction effect of storage time × essential oil separation treatment on carotenoid content of *Nigella sativa* oil. The means with different letters (a–d) are significantly different at $p \leq 0.05$. OWE: oil with essential oil, OWOE: oil without essential oil. (ns and **: non-significant and significant at $p \leq 0.01$, respectively).

3.4. Thymoquinone Content

Thymoquinone is one of the important bioactive components present in BO with many positive health effects. BO with EO contained a high level of thymoquinone (2856.2 mg/kg oil), but stripping of EO from BO could significantly reduce the thymoquinone content (510.6 mg/kg oil) (Figure 6). Thymoquinone, being a monoterpene, is a volatile compound compared to fixed oils; therefore, it can be separated and stripped during steam separation, and its content can be reduced.

Thymoquinone was also reduced significantly ($p < 0.05$) in the oil with EO during storage (Figure 6). However, this reduction was not significant in the oil without EO (Figure 6). It has been reported that thymoquinone could be decomposed and reduced during storage [15]. Thymoquinone reduction via EO separation could decrease BO nutritional properties, as it is one of the valuable bioactive compounds [16,27].



Source of Variation	df	MS	F	p-Value
Storage time	3	511,513.0	16.03 **	0.0002
Essential oil separation	1	21,603,037.5	676.83 **	0.0001
Essential oil separation × storage time	3	309,638.5	9.70 **	0.0007
Error	16	31,917.87		
CV (%)			13.17	

Figure 6. The interaction effect of storage time × essential oil separation treatment on thymoquinone content of *Nigella sativa* oil. The means with different letters (a–e) are significantly different at $p \leq 0.05$. OWE: oil with essential oil, OWOE: oil without essential oil. (**: significant at $p \leq 0.01$).

3.5. Rancimat Analysis

The Rancimat method can be used to determine oxidative stability, which can be helpful to understand and assess the stability of fats and oils, along with other quality parameters and experiments such as PV and AV. Also, the Rancimat method is faster than some other experiments, such as the Schaal test, which can take several days to determine the oxidative stability of oil samples [28]. Rancimat results depend on the fatty acid composition, as well as the presence of antioxidants and prooxidants in the oil samples [29]. Vegetable oils with low oxidative stability, about 8 (h) in the Rancimat test, are suitable for use as salad oil; however, vegetable oils with high oxidative stability, such as 12 (h), can be used for cooking as well. Rancimat resistance above 15 (h) suits intense thermal processes such as frying [11]. The results of the Rancimat test of the samples at 110 °C showed that BO without EO was more stable (8.5 h) than the oil with EO (8.0 h). This action may indicate the effect of extracting the EO from the oil.

3.6. Total Phenolic Content

Phenolic compounds are known to be an important factor in the evaluation of oil quality, as they indicate nutritional, organoleptic and antioxidant properties in oil [30]. Stripping of the EO could significantly reduce the total phenol content in BO (Table 1). It should be mentioned that the classical spectrophotometric analysis of polyphenol content with the Folin–Ciocalteu reagent reveals an increase in the total phenolic content during storage (Table 1). The Folin–Ciocalteu reagent, although widely used, is not specific and detects all phenolic groups. Thus, the increase in the phenolic fraction of the examined samples is probably due to the fact that degradation of complex phenolic complex reactions occurs during storage, and the newly generated components can react with the Folin–

Ciocalteau reagent, causing an increase in the measured phenolic content (Table 1). Similar results have been reported regarding the increase in the amount of phenolic compounds with increasing storage time of black seed oil [16].

Table 1. The interaction effect of storage time $\times$ essential oil separation treatment on total phenol content (mg/kg oil) of *Nigella sativa* oil.

	Day 1		Day 90	
	OWE	OWOE	OWE	OWOE
Total Phenolic Content	128.6 $\pm$ 2.7 b	62.4 $\pm$ 3.1 c	225.1 $\pm$ 5.8 a	118.3 $\pm$ 4.9 b
Source of variation	df	MS	F	p-value
Storage time	1	16,074.72	79.11 **	0.0008
Essential oil separation	1	22,429.45	110.39 **	0.0003
Essential oil separation $\times$ storage time	1	1559.52	7.67 *	0.0243
Error	8	203.18		
CV (%)			11.42	

The means with different (a–c) letters are significantly different at $p \leq 0.05$. OWE: oil with essential oil, OWOE: oil without essential oil. (* and **: significant at $p \leq 0.05$ and $p \leq 0.01$, respectively).

3.7. Phytosterol Composition

Phytosterols are minor components which present in vegetable oils in different amounts. They are important from nutritional and technological points of view [1]. Phytosterols are considered the unsaponifiable matter in vegetable oils with a complex composition that constitutes, on average, 0.3–2% of the oil. Phytosterols are a fingerprint in vegetable oils, can provide rich information about their identity, and can be used to detect vegetable oil adulteration even better than fatty acids profile [31].

The main phytosterol detected in BO was β -sitosterol, followed by stigmasterol, campesterol, Delta 5-Avenasterol and other minor phytosterols (Table 2). All phytosterols were reduced by EO stripping process, and the total phytosterol content was reduced at a level of 11% during EO stripping (Table 2). EO stripping is similar to the deodorization step, during which phytosterols can be separated and reduced. The obtained results showed that the phytosterols were decreased at a lower rate during storage compared to the EO stripping stage (Table 2). There are many reported data showing that phytosterols are less sensitive to storage and less reduced during storage at room conditions [32].

Table 2. Change in phytosterols (mg/kg oil) of black cumin oil with essential oil (OWE) and without essential oil (OWOE) during storage.

Sterol	Day 1		Day 90	
	OWE	OWOE	OWE	OWOE
Cholesterol	52.3 $\pm$ 1.56 a	20.1 $\pm$ 0.40 c	25.1 $\pm$ 0.50 b	19.8 $\pm$ 0.39 c
Campesterol	360.3 $\pm$ 18.01 a	326.3 $\pm$ 16.31 b	366.0 $\pm$ 18.3 a	300.1 $\pm$ 15 c
Stigmasterol	503.9 $\pm$ 25.19 a	439.2 $\pm$ 17.56 b	502.0 $\pm$ 25.1 a	406.7 $\pm$ 16.26 c
β -sitosterol	1503.2 $\pm$ 105.22 a	1330.3 $\pm$ 79.81 b	1442.3 $\pm$ 100.96 b	1319.6 $\pm$ 92.37 b
Delta 5 Avenasterol	332.1 $\pm$ 9.96 a	303.7 $\pm$ 12.14 b	262.6 $\pm$ 7.87 c	252.0 $\pm$ 7.56 c
Delta 7 Stigmasterol	20.3 $\pm$ 0.40 a	19.70 $\pm$ 0.39 b	16.8 $\pm$ 0.33 d	17.5 $\pm$ 0.35 c

Table 2. Cont.

Sterol	Day 1		Day 90		
	OWE	OWOE	OWE	OWOE	
Delta 7 Avenasterol	72.8 ± 2.18 a	67.6 ± 2.02 c	69.8 ± 2.09 b	64.9 ± 1.94 c	
Total sterols	2815 ± 253.35 a	2510 ± 225.9 b	2794 ± 251.46 a	2480 ± 223.2 b	
Sterol	Source of variation	df	MS	F	p-value
Cholesterol	Storage time	1	568.56	1242.76 **	0.0004
	Essential oil	1	1064.08	2325.86 **	0.0001
	Essential oil × storage time	1	546.75	1195.08 **	0.0005
	Error	8	0.46		
	CV (%)			2.30	
Campesterol	Storage time	1	216.75	2.64 ^{ns}	0.1426
	Essential oil	1	5764.08	70.29 **	0.0001
	Essential oil × storage time	1	234.08	2.85 ^{ns}	0.1296
	Error	8	82		
	CV (%)				
Stigmasterol	Storage time	1	645.33	2.78 ^{ns}	0.1335
	Essential oil	1	24,843	107.35 **	0.0001
	Essential oil × storage time	1	1160.33	5.01 ^{ns}	0.0555
	Error	8	231.42		
	CV (%)			3.31	
β-sitosterol	Storage time	1	14,840.33	4.52	0.0661
	Essential oil	1	88,408.33	26.98	0.0008
	Essential oil × storage time	1	22,881.33	6.97	0.0297
	Error	8	3280.08		
	CV (%)			4.07	
Delta 5 Avenasterol	Storage Time	1	10,920.33	293.16	0.0001
	Essential oil	1	1121.33	30.1	0.0006
	Essential oil × storage time	1	243	6.52	0.0340
	Error	8	37.25		
	CV (%)			2.14	
Delta 7 Stigmastenol	Storage time	1	23.80	921.32 **	0.0001
	Essential oil	1	0.001	0.03 ^{ns}	0.9999
	Essential oil × storage time	1	0.701	27.13 **	0.0008
	Error	8	0.026		
	CV (%)			0.87	
Delta 7 Avenasterol	Storage time	1	19.25	12.63 **	0.0075
	Essential oil	1	99.76	65.42 **	0.0001
	Essential oil × storage time	1	0.56	0.37 ^{ns}	0.9999
	Error	8	1.52		
	CV (%)			1.78	
Total sterols	Storage time	1	1386.75	0.18 ^{ns}	0.9999
	Essential oil	1	480,400.08	60.87 **	0.0001
	Essential oil × storage time	1	47,000.08	5.95 *	0.0405
	Error	8	7892.75		
	CV (%)			3.42	

The means with different letters (a–d) are significantly different at $p \leq 0.05$. (ns, * and **: non-significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively).

3.8. Tocopherols Content

Tocopherols are natural antioxidants. This essential nutrient occurs naturally in many foods. Tocopherols consist of four different forms, including α-, β-, γ-, and δ-tocopherol.

Four forms of tocopherols have been found in BO with different proportions. Different types of tocopherols were reduced via EO separation, but reduction in β - and δ -tocopherols was at a higher rate compared to α - and σ -tocopherols (Table 3). Also, it has been shown that steam stripping and vegetable oil storage can reduce tocopherols [33], and the obtained results in this study are in agreement with those reports.

Table 3. Change in tocopherol composition and content (mg/kg oil) of black cumin oil with essential oil (OWE) and without essential oil (OWOE) during storage.

Tocopherol	Day 1			Day 90	
	OWE	OWOE		OWE	OWOE
α -Tocopherol	38.4 $\pm$ 1.15 a	31.0 $\pm$ 0.93 c		36.6 $\pm$ 1.09 b	29.8 $\pm$ 0.89 d
β -Tocopherol	9.7 $\pm$ 0.19 a	1.3 $\pm$ 0.02 d		5.8 $\pm$ 0.11 b	1.5 $\pm$ 0.03 c
γ -Tocopherol	136.3 $\pm$ 6.81 a	87.4 $\pm$ 3.49 c		100.4 $\pm$ 4.01 b	44.5 $\pm$ 1.33 d
δ -Tocopherol	4.5 $\pm$ 0.09 a	3.9 $\pm$ 0.07 b		2.2 $\pm$ 0.04 d	2.8 $\pm$ 0.05 c
Tocopherol	Source of variation	df	MS	F	<i>p</i> -value
α -Tocopherol	Storage time	1	8.33	33.67 **	0.0004
	Essential oil	1	149.81	605.31 **	0.0001
	Essential oil $\times$ storage time	1	0.03	0.12 ^{ns}	0.9899
	Error	8	0.247		
	CV (%)			1.47	
β -Tocopherol	Storage time	1	10.30	1622.76 **	0.0005
	Essential oil	1	123.52	19,452.12 **	0.0001
	Essential oil $\times$ storage time	1	12.73	2004.85 **	0.0004
	Error	8	0.006		
	CV (%)			1.73	
γ -Tocopherol	Storage time	1	4496.94	807.35 **	0.0001
	Essential oil	1	8764.21	1573.47 **	0.0001
	Essential oil $\times$ storage time	1	20.02	3.59 ^{ns}	0.0946
	Error	8	5.57		
	CV (%)				
δ -Tocopherol	Storage time	1	9.08	5359.72 **	0.0000
	Essential oil	1	0.001	0.71 ^{ns}	0.9998
	Essential oil $\times$ storage time	1	1.20	714.85 **	0.0001
	Error	8	0.002		
	CV (%)				

The means with different letters (a–d) are significantly different at $p \leq 0.05$. (ns and **: non-significant and significant at $p \leq 0.01$, respectively).

3.9. Fatty Acid Profile Analysis

Fatty acid analyses are an important factor in determining the nutritional and technological properties of vegetable oils. Also, this analysis can help to monitor changes in fatty acid composition during processing and storage. The fatty acid composition of BO includes linoleic acid (58.04%) as the main fatty acid, followed by oleic (21.9%) and palmitic acids (12.8%) [15]. Vegetable oils with a high content of linoleic acid, a polyunsaturated fatty acid found in BO, have lower oxidative stability compared with vegetable oils with high content of saturated fatty acids [7]. Fatty acid profile analysis of BO with EO and without EO did not show significant changes during EO separation (Table 4).

Table 4. Change in fatty acid (%) of black cumin oil with essential oil (OWE) and without essential oil (OWOE) during storage.

Fatty Acid	Day 1		Day 90		
	OWE	OWOE	OWE	OWOE	
C16:0 (Palmitic acid)	13.2 ± 0.52 c	13.4 ± 0.40 c	13.7 ± 0.41 b	13.9 ± 0.55 a	
C18:0 (Stearic acid)	3.5 ± 0.07 d	3.8 ± 0.11 c	4.0 ± 0.08 b	4.2 ± 0.08 a	
C18:1 (Oleic acid)	24.8 ± 0.74 b	25.8 ± 1.03 a	25.1 ± 1.00 b	25.9 ± 1.03 a	
C18:2 (Linoleic acid)	54.3 ± 2.71 a	54.0 ± 2.16 a	52.0 ± 2.60 b	52.1 ± 2.60 ab	
C22:0 (Behenic acid)	2.5 ± 0.05 b	2.5 ± 0.05 b	2.4 ± 0.07 c	2.7 ± 0.05 a	
Fatty acid	Source of variation	Df	MS	F	<i>p</i> -value
C16:0 (Palmitic acid)	Storage time	1	1.61	43.02 **	0.0002
	Essential oil	1	0.33	8.89 *	0.0176
	Essential oil × storage time	1	0.05	1.42 ^{ns}	0.2672
	Error	8	0.04		
	CV (%)			1.42	
C18:0 (Stearic acid)	Storage time	1	0.59	294.82 **	0.0000
	Essential oil	1	0.19	96.27 **	0.0000
	Essential oil × storage time	1	0.0001	0.05 ^{ns}	0.9999
	Error	8	0.002		
	CV (%)			1.15	
C18:1 (Oleic acid)	Storage time	1	0.01	0.05 ^{ns}	0.9999
	Essential oil	1	5.88	21.06 **	0.0018
	Essential oil × storage time	1	0.03	0.11 ^{ns}	0.9998
	Error	8	0.28		
	CV (%)			2.09	
C18:2 (Linoleic acid)	Storage time	1	20.02	10.08 *	0.013
	Essential oil	1	1.14	0.57 ^{ns}	0.9999
	Essential oil × storage time	1	5.20	2.62 ^{ns}	0.1443
	Error	8	1.98		
	CV (%)			2.67	
C22:0 (Behenic acid)	Storage time	1	0.009	6.24 *	0.0370
	Essential oil	1	0.046	33.39 **	0.0004
	Essential oil × storage time	1	0.059	43.02 **	0.0002
	Error	8	0.001		
	CV (%)			1.46	

The means with different letters (a–d) are significantly different at $p \leq 0.05$. (ns, * and **: non-significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively).

Fatty acids are located in the triacylglycerol (TAG) structure in vegetable oils. TAGs have high molecular weight, so they are not stripped in the EO separation step. This explains the stability of fatty acids during EO separation. However, linoleic acid (18:2) was reduced in both oils during storage, which can be due to oxidation [34]. It has been shown that the fatty acid profile of vegetable oils can be changed during storage [35].

4. Conclusions

Despite the different applications of black cumin seed oil in the food, pharmaceutical and cosmetic industries, this oil does not have good stability and has a high AV and PV immediately after extraction. The separation of EO by hot steam was a new and successful method in EO extraction without increasing the primary oxidation of the oil. The profile of fatty acids did not change significantly during EO stripping. AV and PV increased in

all samples, but the increase in samples without EO was much less during storage. The amount of chlorophylls and tocopherols in the samples decreased during storage, but the decrease was smaller in the sample without EO. Also, the oil sample without EO showed more resistance in the Rancimat test. Therefore, EO separation of BO by steam stripping can be used as an efficient method to reduce oxidation and increase shelf life. Vacuum extraction of edible oils in industry can have many applications, such as its use in refining and increasing the shelf life of other edible oils, such as sunflower oil, sesame oil, etc., as well as oils with therapeutic and medicinal properties, such as olive oil, by reducing the effects of degradation and negative properties. Also, the separated essential oils can be recycled and collected, which, due to the short extraction time and the vacuum of the system, reduces the degradation of the essential oil and even increases the efficiency of extraction. It should be noted that stripping could relatively decrease the EO and bioactive components, but enhance the oxidative stability of BO. However, these components remain in pretreated BO in relatively good amounts compared with other common vegetable oils, which can give the BO its functional properties during different applications.

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