



#### Crescent Journal of Medical and Biological Sciences

Vol. 12, No. 4, October 2025, 201–207 eISSN 2148-9696

# Impact of Beta-Cyclodextrin Treatment on Medicinal Constituents and Quality Indices of *Nigella sativa* Seed Oil



doi 10.34172/cjmb.2025.3900

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#### **Abstract**

Objectives: Black cumin (Nigella sativa L.) seeds (BS) contain approximately 25%–35% oil, which has widespread applications in the food, pharmaceutical, and cosmetic industries. Compared to other vegetable oils, BS oil tends to have higher acid and peroxide values (PVs), reducing its applications. Different pre-treatments of black cumin seeds were used to enhance the quality of extracted oil, but they were not very effective. Applying adsorbents such as  $\beta$ -cyclodextrin is gaining attention to reduce the impurities from fats and oils. Therefore, the present research aims to examine the impact of using  $\beta$ -cyclodextrin as an adsorbent in the processing of BS oil.

Materials and Methods: This study investigates the effects of pretreatment of BS oil with various concentrations (0 (control sample), 2.5, 5, 7.5 and 10%) of  $\beta$ -cyclodextrin dissolved in water: ethanol at ratios of 1:1 or 1:2 (v/v) on thymoquinone, chlorophyll, carotenoids, peroxide value (PV) and acid value (AV), oxidative stability and fatty acids. High-performance liquid chromatography and spectrometry determined thymoquinone content, chlorophyll, and carotenoid amount. PV and AV were measured by titration. Oxidative stability was determined using the Rancimat equipment. Fatty acid composition was also determined by gas chromatography.

**Results:** The results revealed that  $\beta$ -cyclodextrin, up to 5% dissolved in water: ethanol (1:2, v/v), could significantly reduce the PV (16.9 to 10.2 meq  $O_2$ /kg oil) and AV (4.2 to 2.7 mg KOH/g oil) and increase the oxidative stability (9.8 to 12.7 h). Moreover,  $\beta$ -cyclodextrin could also reduce the bioactive components (thymoquinone and carotenoids). Still, these reductions were smaller when the oil samples were treated with  $\beta$ -cyclodextrin at concentrations below 7.5%. However, pretreatment of BS oil with  $\beta$ -cyclodextrin did not affect the fatty acid composition of the oil samples.

Conclusions: Overall, the findings suggest that  $\beta$ -cyclodextrin treatment (up to 5%) can be an effective strategy to enhance the stability of black seed oil, expanding its potential applications in the food, pharmaceutical, and cosmetic industries.

Keywords: Fatty acids, Nigella sativa oil, Physicochemical properties, Quality, Stability

## Introduction

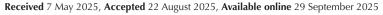
Nigella sativa L., commonly known as black cumin, is an annual herb from the Ranunculaceae family. Its seeds, referred to as black cumin seeds (BS), contain approximately 25%–35% oil, which has widespread applications in the food, pharmaceutical, and cosmetic industries. Numerous studies have demonstrated the oil's therapeutic activities, including antihypertensive, antidiabetic, antimicrobial, anticancer, anti-inflammatory, and antioxidant properties (1,2).

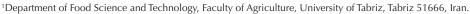
Previous studies on raw BS oil have identified it as a substantial source of essential fatty acids, tocopherols, phytosterols, polyphenols, essential oils, and other bioactive compounds. Thymoquinone, one of the major bioactives found in the essential oil fraction, offers a range of health benefits and distinguishes BS oil from different vegetable oils. It exhibits medicinal properties such as strong anticancer and antioxidant effects (3).

BS oil is typically extracted via cold pressing or occasionally with solvents such as hexane. Cold-pressed

oil, if of sufficient and suitable quality, requires no refining and retains its bioactive compounds and nutritional value more effectively. Solvent-extracted oil, however, may need refining to eliminate impurities and improve quality (4-6). Compared to other vegetable oils, BS oil tends to have higher acid and peroxide values (PVs) at the time of extraction and during storage. Elevated acid value (AV), which means high free fatty acid content, can reduce the smoke point and decrease its usability in food preparation, especially frying and cooking. Free fatty acids also exhibit higher oxidation susceptibility than esterified fatty acids in triacylglycerol molecules. A higher PV also shows low oil quality. It means that the oil has a high content of oxidation products, which can also reduce the oil's application in food, pharmaceutical, and cosmetic products (1).

Oils with high acid and PVs require refining processes to become suitable for consumption. While refining helps eliminate undesirable compounds and prolongs shelf life, it may also reduce nutritional value and antioxidant activity. It can also reduce the oil's health-promoting





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bioactive components (6). The impacts of refining stages on oil quality have also been explored. Given that the beneficial properties of BS oil are mainly attributed to its non-glyceride and bioactive components—especially thymoquinone—refining may lead to the removal of these compounds and diminish the oil's functionality (6). Due to these changes during refining and the reduction of bioactive compounds, finding alternative approaches for processing and optimizing BS oil refining has become a research focus.

Various pretreatment methods—including microwave irradiation, roasting, and adjusting the moisture and pH of BS before oil extraction—have been employed to lower the acid and PVs in extracted black seed oil (7,8). Blending of extracted BS oil with other vegetable oils has been used to balance the oxidative stability and peroxide and AVs (9,10). Also, these approaches were relatively practical in controlling oxidation and preserving extracted oil quality; however, one new potential approach can be using selective adsorbents to remove peroxidative substances from the oil.

β-Cyclodextrin has gained attention as an effective adsorbent for removing certain impurities and improving oil quality (11). It is a cyclic oligosaccharide with seven glucose units linked via α-1,4 glycosidic bonds. Structurally,  $\beta$ -cyclodextrin resembles a truncated cone, featuring a hydrophobic internal cavity and a hydrophilic outer surface due to hydroxyl groups. Chemically, it acts as a molecular host that can selectively incorporate other molecules into its matrix via non-covalent interactions through its hydrophobic cavity or surface hydroxyl groups.

Figure 1 illustrates the chemical and three-dimensional structure of  $\beta$ -cyclodextrin. The molecule has a larger cavity side (approximately 6.0-6.5 Å in diameter) and a smaller cavity side (approximately 4.7-5.3 Å), with a height of around 7.8 Å (12).

It has been shown that  $\beta$ -cyclodextrin can remove and reduce some health-related components, such as cholesterol, from lard and shrimp oil, which could enhance oil quality (11,13).

Peroxides and free fatty acids are the main concerns in fresh-extracted BS oil and play a leading role in limiting its application in different fields. The present research aims to examine the impact of using  $\beta$ -cyclodextrin

as an adsorbent in the processing of BS oil. The results can improve understanding of how different processing approaches affect oil quality and support the broader utilization of this health-promoting oil.

#### Materials and Methods

#### Materials

Black cumin seed (Nigella sativa) was purchased from the local market in Tabriz, Iran. β-Cyclodextrin was obtained from Merck (USA). All solvents and chemical reagents, including chloroform, acetic acid, potassium iodide, starch, phenolphthalein, ethanol, Folin-Ciocalteu reagent, and sodium carbonate, were sourced from Sigma-Aldrich (Germany).

#### Oil Extraction

Oil was extracted from cleaned and sieved BS using a screw press (model P500R, Anton Fries, Germany) (7). The crude oil was filtered through cloth filters to remove residual seed particles and stored in a refrigerator at 4 °C for later experimental use.

# Pre-treatment With β-Cyclodextrin

Extracted oil of BS by cold press was pretreated with β-cyclodextrin according to the previously described method with slight modification (14). β-Cyclodextrin was dissolved in a 1:1 (v/v) or 1:2 (v/v) mixture of water: ethanol. Initially, β-cyclodextrin was dissolved in water under continuous stirring, followed by adding ethanol at the mentioned ratios. The resulting solutions from both ratios were separately added to BS oil to obtain final concentrations of 2.5%, 5%, 7.5%, and 10% β-cyclodextrin. The mixtures were stirred thoroughly at 30 °C for three hours. After mixing, the obtained samples were centrifuged at 5000 g to separate the aqueous phase from the oil phase. The oil phase was stored in opaque glass bottles at ambient temperature until further analysis. A control sample without  $\beta$ -cyclodextrin treatment was also prepared and stored under the same conditions for comparison.

#### Acid Value

The AV of the extracted oil was measured using the AOCS official method (15). For this test, 10 g of oil was mixed with

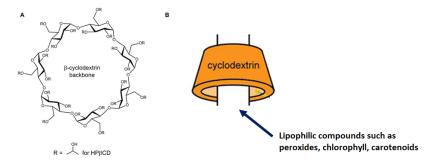


Figure 1. Chemical Structure of the β-Cyclodextrin Showing its Interaction Sites With Different Chemicals.

100 mL of a 50:50 (v/v) neutralized ethanol–chloroform mixture. Three to four drops of phenolphthalein indicator were added, and titration was conducted using 0.01 N potassium hydroxide until a persistent pale pink color appeared (at least 30 seconds). AV was calculated using the following formula:

$$AV = (V \times N) \times 56.1 / W$$

where V is the volume (mL) of KOH solution used, N is the normality, and W is the weight of the oil sample in grams.

#### Peroxide Value

PV was measured using the AOCS Cd 8-53 method (15). A 5 g oil sample was mixed with 30 mL of an acetic acid–chloroform solution (2:3 v/v), followed by 0.5 mL of saturated potassium iodide solution. The mixture was sealed and left in the dark for one minute. Then, 30 mL of distilled water and 0.5 mL of starch indicator were added, and the sample was titrated with 0.1 N sodium thiosulfate. A blank sample (without oil) was run simultaneously. PV was calculated using the following formula:

$$PV = (a - b) \times N \times 1000 / m$$

where a is the volume of titrant used for the sample, b is the volume used for the blank, N is the normality of sodium thiosulfate, and m is the oil weight (g).

## Oxidative Stability Assessment

The oxidative stability of BS oil samples was measured using a Rancimat device (model 734, USA) at 110 °C and an air flow rate of 20 L/h, following the method described by Wagner et al (16).

# Carotenoid Content

7.5 g of oil was diluted to 25 mL with cyclohexane in a volumetric flask to determine carotenoid content. Absorbance was measured at 470 nm using a spectrophotometer, and carotenoid content was calculated in mg/kg of oil using the following formula (17):

Carotenoid (mg/kg) =  $A_{470} \times 10^6$  / (2000 × 100 × d) where  $A_{470}$  is the absorbance at 470 nm, d is the path length (1 cm), and 2000 is the extinction coefficient of lutein.

## Chlorophyll Content Measurement

Similarly, after preparing the oil solution with cyclohexane, the chlorophyll content was determined spectrophotometrically at a wavelength of 670 nm (17). The content was calculated using the following formula:

$$Chlorophyll(mg/kg) = \frac{(\text{A670} \times 10^6)}{(613 \times 100 \times d)}$$

A670 = absorbance at 670 nm d = spectrophotometer cell thickness (1 cm)

# Fatty Acid Analysis

Fatty acid methyl esters were prepared by mixing oil with hexane and reacting with 7 mL of 2 N methanolic potassium hydroxide at 50 °C for 20 minutes. Analysis was performed using a gas chromatograph (ACM6000, USA) with Autochrom2000 software using the previously published method (18). The instrument had a BPX70 capillary column (silica, 120 m length, 0.22 mm inner diameter, 0.2 µm film thickness) and a flame ionization detector (FID). Carrier gas (helium) flowed at 17 mL/min, air at 300 mL/min, and hydrogen at 30 mL/min. Oven, injector, and detector temperatures were set at 198 °C, 250 °C, and 280 °C, respectively. Fatty acids were identified by comparing retention times with standard references and reported as relative percentages of peak areas.

#### Thymoquinone Quantification

Thymoquinone content was measured using high-performance liquid chromatography (HPLC). Oil samples were passed through a pre-rinsed C18 column with methanol. A 20  $\mu$ L oil sample, followed by two 400  $\mu$ L volumes of methanol, was used for cleaning. Analysis was performed using an Agilent 1200 HPLC system with a diode array detector (DAD). Separation was achieved on a Prevail C18 column (4.6 mm  $\times$  250 mm, 5  $\mu$ m particle size, Agilent Technologies, USA) with a mobile phase of water: methanol:2-propanol (50:45:5 v/v), filtered through a 0.45  $\mu$ m Millipore membrane. Flow rate was 1.5 mL/min, injection volume 20  $\mu$ L. Identification was confirmed by matching retention times with standard thymoquinone. Quantification was achieved using linear calibration curves (3,19).

## Statistical Analysis

Research was conducted using a completely randomized design. All tests were performed in triplicate. Mean values were calculated, and data were analyzed via ANOVA. Significant differences among means were identified using Duncan's multiple range test at a 5% significance level with SPSS software.

#### **Results and Discussion**

## Solubility of β-Cyclodextrin

Due to its chemical structure,  $\beta$ -cyclodextrin is highly soluble in water but less soluble in ethanol (12). Increasing the ratio of ethanol in a water–ethanol solvent mixture reduced its solubility, and at ratios greater than 2:1 (ethanol to water), a gummy phase formed, indicating incomplete dissolution. This is because ethanol, an organic solvent, hinders the solubility of  $\beta$ -cyclodextrin, which is more hydrophilic. Additionally, higher ethanol concentrations interfered with phase separation during centrifugation, leading to difficulties isolating aqueous and oil phases. Therefore, mixtures with ethanol-to-water ratios of 1:1 and 2:1 were used, as higher ethanol ratios impeded the functional application of  $\beta$ -cyclodextrin in this study.

#### Acid and Peroxide Values

The AV, which reflects free fatty acid content in edible oils, serves as a quality index for triacylglycerol hydrolysis due to enzymes such as lipase or poor handling of oilseeds (e.g., immature, damaged, or improperly stored seeds) (17). Freshly extracted BS oil showed a high AV, likely due to active lipase enzymes, and this value increased over storage time (7). The results showed that this index can be reduced by pre-treatment with  $\beta$ -cyclodextrin (Table 1). Higher concentrations of β-cyclodextrin were also significantly effective in reducing the AV (Table 1). This can be explained by the fact that the hydrocarbon chain of free fatty acids can fit into the hydrophobic cavity of β-cyclodextrin. In contrast, the carboxylic acid group may form hydrogen bonds with the hydroxyl groups on its outer surface (12).

Peroxide formation, indicative of oxidative degradation, occurs under improper storage conditions such as elevated temperature, moisture, or oxidative stress. Peroxides elevate the PV, lowering oil quality (3,6). Results showed that β-cyclodextrin treatment significantly reduced PV, especially at higher concentrations and ethanol ratios (Table 1). Hydrophobic portions of peroxide molecules can enter the cyclodextrin cavity, while polar groups may bond to the exterior hydroxyls through hydrogen bonding. Higher ethanol ratios appeared to enhance the solubility of peroxides and free fatty acids, increasing their availability for complexation with β-cyclodextrin and thus reducing their concentration in the oil phase. Previously published data also show that  $\beta$ -cyclodextrin treatment can reduce peroxides and other shrimp oil oxidation products (11). Oxidative stability of the BS oil is affected by the raw

material (seeds) used for oil extraction, the fatty acid composition, and minor components present in the extracted oil (6). The results showed that the oxidative stability of the pre-treated oils with  $\beta$ -cyclodextrin dissolved in water: ethanol at a ratio of 1:2 (v/v) at a concentration of up to 7.5% was significantly increased (from 9.8 to 12.7 h). Also, results showed that the amount of ethanol used to dissolve the  $\beta$ -cyclodextrin significantly affected the removal of peroxides and free fatty acids, which could positively affect oxidative stability (Table 1).

#### Carotenoids

Carotenoids are bioactive compounds contributing to oxidative stability and nutritional value. Reported levels in raw black seed oil range from 3 to 6.57 mg/kg (6,8), while roasted seeds yielded 8.6 mg/kg (7). Solvent extraction generally produces higher carotenoid content than cold pressing (6).

Treatment with β-cyclodextrin caused a significant decrease in carotenoids, especially at 10% concentration dissolved in high-ethanol solvent, reducing levels from 4.8 mg/kg in untreated oil to 2.5 mg/kg (Table 2). The hydrophobic nature of carotenoids facilitates their encapsulation by  $\beta$ -cyclodextrin. Ethanol enhances their solubility, promoting complex formation and removal from oil.

Notably, no significant reduction occurred at β-cyclodextrin concentrations higher than 7.5%, suggesting a plateau effect. Given the nutritional importance of carotenoids, optimal solvent ratios should be selected to minimize loss during treatment. Carotenoids can act as antioxidants in the stability of vegetable oils and

Table 1. Effects of Pretreatment of Nigella sativa Seed Oil With β-Cyclodextrin on its Oxidative Quality

Properties	Control sample*	T2.5		Т5		T7.5		T10	
		1:1	1:2	1:1	1:2	1:1	1:2	1:1	1:2
Acid value	4.2 ±0.1 <sup>a**</sup>	3.8 ±0.2 <sup>b</sup>	3.0 ±0.1°	3.1 ±0.1°	2.7 ±0.3 <sup>d</sup>	3.2 ±0.1°	2.8 ±0.1 <sup>cd</sup>	3.0 ±0.2°	2.9 ±0.1 <sup>cd</sup>
Peroxide value (meq O <sub>2</sub> /kg oil)	16.9 ±0.8a	14.0 ±0.1 <sup>b</sup>	11.9 ±0.3°	11.8 ±0.5°	10.2 ±0.7 <sup>d</sup>	10.5 ±0.4 <sup>d</sup>	10.1 ±0.6 <sup>d</sup>	10.7 ±0.5 <sup>cd</sup>	10.2 ±0.3 <sup>d</sup>
Oxidative stability (h)	9.8 ±0.8e	10.1 ±0.1 de	10.5 ±0.1 <sup>d</sup>	12.0 ±1.2 <sup>b</sup>	12.7 ±1.0 <sup>a</sup>	11.8 ±0.5 <sup>b</sup>	11.5 ±0.6°	11.3 ±0.8 °	11.6 ±1.2bc

<sup>\*</sup>Control sample: Nigella sativa seed oil without any pretreatment. T2.5 to T10: pretreatment of Nigella sativa oils with \(\beta\)-cyclodextrin at 2.5 to 10%, respectively, prepared by dissolving in water: ethanol at a ratio of 1:1 or 1:2.

Table 2. Effects of Pretreatment of Nigella sativa Seed Oil With β-Cyclodextrin on its Pigment Content (mg/kg oil)

Oil	Control	T2.5		Т5		T7.5		T10	
	sample*	1:1	1:2	1:1	1:2	1:1	1:2	1:1	1:2
Chlorophyll	1.7 ± 0.2**a 1.	.6 ± 0.2ª	1.1 ± 0.1 <sup>b</sup>	1.5 ± 0.1 <sup>a</sup>	1.0 ± 0.1 <sup>b</sup>	1.0 ±0.1 <sup>b</sup>	0.5 ± 0.1°	0.9 ± 0.2 <sup>b</sup>	0.4 ± 0.1°
Carotenoids	4.8 ± 0.5 <sup>a</sup> 4.	.3 ± 0.5ab	$2.9 \pm 0.1^{d}$	$4.2 \pm 0.2^{b}$	2.5 ± 0.3°	3.6 ±0.1°	$2.1 \pm 0.4^{e}$	$3.3 \pm 0.2^{cd}$	$2.0 \pm 0.3^{e}$

<sup>\*</sup>Control sample: Nigella sativa seed oil without any pretreatment. T2.5 to T10: pretreatment of Nigella sativa oils with \(\beta\)-cyclodextrin at 2.5 to 10%, respectively, prepared by dissolving in water: ethanol at a ratio of 1:1 or 1:2.

<sup>\*\*</sup> Mean is shown as  $\pm$  SD. Different letters show a significant difference at the level of 5%.

<sup>\*</sup> Mean is shown as ± SD. Different letters show a significant difference at the level of 5%.

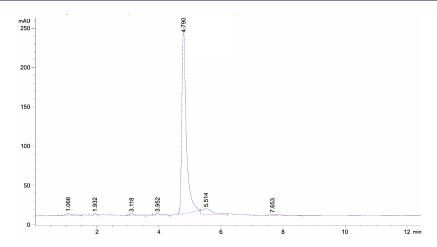


Figure 2. Chromatogram Obtained From the Analysis of Nigella sativa Oil to Detect and Determine the Thymoquinone.

as precursors of vitamin A in the body (6).

# Chlorophyll

Chlorophyll imparts green coloration to oil and can induce oxidation when exposed to light. Its content depends on seed origin, pretreatment, and extraction method (20). For example, oils from Tunisian and Iranian seeds contained 6 and 2.26 mg/kg, respectively. Solvent-extracted oil had higher chlorophyll (16.72 mg/kg) than hot-pressed oil (6.02 mg/kg) (6). Roasting increased chlorophyll content from 3.04 to 5.77 mg/kg (21).

The results showed that chlorophyll content decreased significantly from 1.7 mg/kg in untreated oil to 0.5 mg/kg in treated oil with  $\beta$ -cyclodextrin at a concentration of 7.5% dissolved in water: ethanol (1:2, v/v) (Table 2). Its lipophilic nature promotes complexation and removal, which increases when the water content in the solvent decreases. However, higher  $\beta$ -cyclodextrin concentrations beyond 7.5% did not lead to further significant reductions.

# Thymoquinone

Thymoquinone (TQ) (2-Isopropyl-5-methyl-1,4-benzoquinone) is a monoterpene with molecular formula  $C_{10}H_{12}O_2$  and molar mass of 164.2 g/mol. Structurally, TQ consists of a six-carbon quinone ring, a methyl group at position C2, and a propyl group at C5. TQ constitutes 30–48% of BS essential oil (22). TQ is an important medicinal component of the *N. sativa* seed and oil extracted from this seed. It exhibits antimicrobial, anti-inflammatory,

anticancer, and antidiabetic properties (23). HPLC is a suitable method to determine the TQ in BS oil, and Figure 2 shows a chromatogram of TQ analysis. Only one main peak is present in the chromatogram obtained from the analysis of TQ via HPLC. It is an advantage of this analysis method in detecting and determining TQ in *N. sativa* oil via this analytical method.

TQ aromatic and polar structure allows stable inclusion in the hydrophobic cavity of β-cyclodextrin, with keto and methoxy groups forming hydrogen bonds with the outer hydroxyls. Ethanol enhances TQ solubility and thus its interaction with β-cyclodextrin, resulting in greater removal from the oil phase (Table 3). TQ was reduced significantly via pretreatment of BS oil with  $\beta$ -cyclodextrin at a concentration of up to 7.5% (Table 3). TQ removal with this pretreatment is a weak point of this approach, and it should be at a lower level as much as possible. Therefore, pretreatment with  $\beta$ -cyclodextrin should be optimized to significantly reduce the peroxides and free fatty acids with low impact on the bioactive and positive health practical components such as TQ and carotenoids. Considering this issue, the effective pretreatment with  $\beta$ -cyclodextrin on the BS oil can be at a level of 5%, which is much more effective in reducing the PV and AV and preserving the bioactive components. The results show that treatment of *N. sativa* oil by  $\beta$ -cyclodextrin at optimum concentration preserves the TQ concentration at a suitable level, which is very important from pharmaceutical and nutritional points of view. Therefore, this method can be used as an

**Table 3.** Effects of Pretreatment of Nigella sativa Seed Oil With  $\beta$ -Cyclodextrin on its thymoquinone content (mg/kg oil)

Oil	Control sample*	T2.5		Т5		T7.5		T10	
		1:1	1:2	1:1	1:2	1:1	1:2	1:1	1:2
Thymoquinone	4150 ±134a**	4100 ± 102 <sup>b</sup>	4061± 58°	3829 ± 67 <sup>d</sup>	3570 ± 91e	2904 ± 113f	2180 ± 58 <sup>h</sup>	2874 ± 82g	2105 ± 38i

<sup>\*</sup>Control sample: Nigella sativa seed oil without any pretreatment. T2.5 to T10: pretreatment of Nigella sativa oils with β-cyclodextrin at 2.5 to 10%, respectively, prepared by dissolving in water: ethanol at a ratio of 1:1 or 1:2.

<sup>\*\*</sup> Mean is shown as  $\pm$  SD. Different letters show a significant difference at the level of 5%.

Table 4. Effects of Pretreatment of Nigella sativa Seed Oil With β-Cyclodextrin on its Fatty Acid Composition (%)

Fatty acid	Control sample*	T2.5		T5		T7.5		T10	
		1:1	1:2	1:1	1:2	1:1	1:2	1:1	1:2
Palmitic acid	12.6 ±0.1 <sup>a**</sup>	12.5 ±0.1ab	12.7 ±0.2a	12.4 ±0.1b	12.9 ±0.2a	12.3 ±0.2b	12.2 ±0.1bc	12.0 ±0.1c	12.7 ± 0.1a
Stearic acid	1.7 ±0.1ab	1.9 ±0.1a	1.8 ±0.1a	1.8 ±0.1a	1.5 ±0.1 b	1.7 ±0.1a	1.9 ±0.1a	1.5 ±0.1b	1.3 ±0.1 b
Oleic acid	23.3 ±0.4a	23.5 ±0.6a	23.3 ±0.3a	23.0 ±0.4a	23.8 ±0.8a	23.0 ±0.5a	23.7 ±0.1a	23.4 ±0.5a	23.9 ±0.9a
Linoleic acid	57.9 ±0.1a	57.6 ±0.9 a	57.4 ±2.1a	58.0 ±0.8a	57.0 ±0.9a	57.8 ±1.3a	56.9 ±1.0a	57.4 ±08a	57.5 ±1.7a
Eicosadienoic acid	2.5 ±0.1 ab	2.5 ±0.1 ab	2.8 ±0.2a	2.3 ±0.1b	2.9 ±0.1a	2.4 ±0.1b	2.3 ±0.3b	2.2 ±0.1 b	2.4 ±0.1

<sup>\*</sup>Control sample: Nigella sativa seed oil without any pretreatment. T2.5 to T10: pretreatment of Nigella sativa oils with \(\beta\)-cyclodextrin at 2.5 to 10%, respectively, prepared by dissolving in water: ethanol at a ratio of 1:1 or 1:2.

alternative to the other pre-treatments already used to control PV and AV of this oil.

# Fatty Acid Profile

The fatty acid composition greatly influences vegetable oils' nutritional quality and oxidative stability (24). BS oil contains high levels of linoleic acid (omega-6), an essential fatty acid, and oleic acid (omega-9), a non-essential fatty acid known for cholesterol-lowering and cardioprotective effects (1).

Pretreatment with  $\beta$ -cyclodextrin did not significantly alter the fatty acid composition of BS oil samples (Table 4). This is likely due to the inability of triacylglycerols to fit into the cyclodextrin cavity. Even if interactions occur, the non-selective nature means no preferential extraction or change in fatty acid ratios.

#### **Conclusions**

Nigella sativa seed oil has many applications in the pharmaceutical, cosmetics, and food industries, but its applications are limited due to its high peroxide and AVs. This research showed that it is possible to pre-treat this oil with β-cyclodextrin, enhancing its oxidative stability. Also, the results showed that this pretreatment with suitable amounts of  $\beta$ -cyclodextrin (up to 5%) could preserve medicinal and health-related components such as thymoquinone, which can significantly impact the application and widen its uses in different fields. The results should show that this pre-treatment is effective at a level comparable to seed roasting and microwaving. However, there is a need for further research to determine its effects on the oil quality and the stability of bioactive components during storage.

#### **Authors' Contribution**

Conceptualization: Ziba Abdian.

Data curation: Sodeif Azadmard-Damirchi.

Formal analysis: Ziba Abdian.

Investigation: Sodeif Azadmard-Damirchi. Methodology: Ziba Abdian, Mohammadali Torbati. Project administration: Sodeif Azadmard-Damirchi.

Supervision: Sodeif Azadmard-Damirchi, Mohammadali Torbati.

Validation: Ziba Abdian, Mohammadali Torbati.

Writing-original draft: Ziba Abdian.

Writing-review & editing: Mohammadali Torbati.

#### **Conflict of Interests**

None to declare.

#### **Ethical Issues**

Not applicable.

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<sup>\*\*</sup> Mean is shown as ± SD. Different letters show a significant difference at the level of 5%.

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