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Enrichment and Characterization of Cold-Pressed Nigella Sativa Oil with Thymoquinone: Physicochemical Properties and Antioxidant Potential

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Abstract

This study investigates the physicochemical properties, fatty acid composition, and antioxidant potential of cold-pressed Nigella Sativa oil enriched with thymoquinone. The raw black cumin seeds used in the study exhibited a moisture content of 3.25±0.28%, an oil content of 37.53±0.73%, a protein content of 18.71±0.48%, and an ash content of 5.16±0.47%. The oil yield obtained through the cold press extraction method was 22.33±0.58%. The cold-pressed oil exhibited a free fatty acid content of 8.24±0.30% as oleic acid, a peroxide value of 9.56±0.43 meq O2/kg lipid, an iodine value of 119.27±5.97 I2/100 g, and a saponification number of 204.76±10.66 mg of KOH/g of oil. The oil was found to be rich in unsaturated fatty acids, particularly linoleic and oleic acids, and had a thymoquinone content of 0.28%. In an effort to enhance the thymoquinone content, the essence of Nigella Sativa oil was extracted, yielding a thymoquinone content of 60%. This essence was then added to the Nigella Sativa oil, resulting in an enriched oil with a thymoquinone content of 2.96%. The results indicate that the cold-pressed Nigella Sativa oil enriched with thymoquinone has good

physicochemical properties and antioxidant potential, making it a promising ingredient for nutritional and medicinal applications.

Keywords: *Nigella Sativa Oil, Cold-Press Extraction, Thymoquinone Enrichment, Physicochemical Properties, Antioxidant Potential*

1. Introduction

Historically, the seeds and oil of Nigella sativa, commonly known as black cumin, have been highly valued for their health-promoting properties. They have been extensively studied for their potential benefits in managing metabolic disorders such as diabetes, hypertension, and high cholesterol [1]. Moreover, the scope of research exploring its therapeutic potential for other health conditions is continually expanding [2]. The seeds are often incorporated into various food items like pastries, cheese, pickles, and baked goods for added flavor [3, 4]. Components of black cumin seeds have also been utilized in the creation of functional dietary supplements and cosmetic products. Research has been conducted on the pharmacological properties of the essential, fixed, and cold-pressed oil of black cumin [5, 6]. Black cumin oil (BCO) is known to be rich in essential fatty acids, bioactive sterols, and tocols [7-9]. Thymoquinone (TQ) is the primary compound in black seed that is responsible for its health benefits. A study that analyzed ten different black seed oil products available in Malaysia found a significant 27-fold variation in the thymoquinone content between bottled oil and oil capsules. The researchers suggested that the thymoquinone content in commercially available black seed oil products should be standardized, potentially by enriching the oils with a standard amount of thymoquinone [10]. However, they did not propose a specific concentration of thymoquinone in black seed oil or a recommended daily dosage.

The concentration of thymoquinone in black seed oil products can be influenced by several factors. These include the geographic location where the black seeds are grown [11], the timing of seed harvest [12], the conditions and duration of seed storage prior to processing, and the specifics of the manufacturing process itself [13]. As the seeds mature, reaching their peak at 75 days post-fertilization, there is a consistent increase in the levels of thymoquinone and other active ingredients such as p-cymene, thymohydroquinone, α-thujene, and carvacrol, along with minor components like α-pinene, sabinene, and β-pinene [12]. While thymoquinone and its derivatives are key contributors to the antioxidant properties of black

seed oils, other isomers of thymol such as carvacrol, t-anethole, and 4-terpineol also play a significant role in enhancing the overall antioxidant activity [14].

For optimal preservation of the aroma and efficacy of bulk-purchased black seed, it is recommended to keep the seed in tightly sealed containers. Factors such as exposure to air, elevated temperatures, and light have been identified to influence the stability of the volatile compounds in the seed [15].

The method of cold-pressing, which excludes the use of heat or solvents, is a technique employed in the extraction of seed oils. Black cumin seed oil (BCSO) derived from this process is unrefined, potentially leading to a higher concentration of lipophilic phytochemicals, including natural antioxidants and derivatives of thymoquinone. In our ongoing pursuit to develop edible seed oils abundant in components beneficial to health, this research aimed to: 1) identify the fatty acid composition of Iranian-origin cold-pressed BCSO, 2) quantify the total phenolic content and the concentrations of thymoquinone and its related compounds in these oils, and 3) augment the thymoquinone content in BCSO, resulting in a thymoquinone-enriched BCSO product. The findings from this investigation will contribute to the creation of innovative edible oil products rich in bioactive compounds and thymoquinone.

2. Materials and Methods

2.1. Raw Materials

The black cumin seeds (BCS) were obtained from local plant's shops (Tehran, Iran), followed by cleaning to remove any extraneous materials. In this study, a variety of chemical compounds were utilized, including Sulfuric Acid (H_2S0_4, CAS No. 7664-93-9), Sodium Hydroxide (NaOH, CAS No. 1310-73-2), Ethyl Alcohol (CH_3CH_2OH, CAS No. 64-17-5), Boric Acid (H_3BO_3, CAS No. 10043-35-3), Sodium Thiosulfate ($Na_2O_3S_2$, CAS No. 7772-98-7), Starch Glue reagent ($(C_6H_{10}O_5)_n$, CAS No. 9005-84-9), Methyl Red ($C_{15}H_{15}N_3O_2$, CAS No. 493-52-7), Phenolphthalein ($C_{20}H_{14}O_4$, CAS No. 77-09-8), Hydrochloric Acid (HCl, CAS No. 7647-01-0), Potassium Iodide (KI, CAS No. 7681-11-0), Acetic Acid (CH_3COOH, CAS No. 64-19-7), Chloroform ($CHCl_3$, CAS No. 67-66-3), Methanol (CH_3OH, CAS No. 67-56-1), Folin–Ciocalteu (C_6H_6O, CAS No. 12111-13-6), Gallic Acid ($C_6H_2(OH)_3CO_2H$, CAS No. 149-91-7), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) ($C_{18}H_{12}N_5O_6$, CAS No. 1898-66-4), Kjeldahl tablet (Na_2SO_4, K_2SO_4, TiO_2, $CuSO_4$), and Hanus Solution (BrI, CAS No. 7789-33-

5). All chemicals were analytical grade and obtained from Merck and Sigma-Aldrich representatives.

2.2. Chemical Composition

To measure moisture, ash, fat, and protein content of BCS, several methods were employed. The American Oil Chemists' Society (AOAC) method No. 925-09 [16] was used to determine moisture, while ash content was measured using the AOAC method No. 968/08 [16]. Fat content was determined using the Iranian National Standard method No. 7593, and protein content was measured using the American Association of Cereal Chemists (AACC) method No. 46-13 [17] with a nitrogen conversion factor (NCF) of 6.25.

2.3 The Extraction of Nigella Sativa Oil

For cold pressing, mechanical oil extraction device (Iran Cold Pressing) was used at room temperature without thermal treatment for 1 kg of whole grain added to the device, then the oil was kept in the dark for one day for oil phase separation. The efficiency percentage was calculated by dividing the weight of the extracted oil by the weight of the seed.

2.4. Physicochemical Composition of Nigella Sativa Oil

The acidity of the sample was assessed by measuring the quantity of free fatty acid based on the Iranian National Standard No. 8617. A solution was made by dissolving the sample in a mixture of diethyl ether and ethanol, which was then titrated in the presence of 1% phenolphthalein reagent with a standardized 0.1 M NaOH. The solution was continuously mixed until a pale and stable pink color was formed in the solution for one minute. The percentage of free fatty acid was expressed in terms of oleic acid in this study.

The determination of peroxide number was conducted by following the AOCS method No. Cd 8b-90 [18]. In this method, 5 g of the oil sample was mixed with a solution of acetic acid and chloroform, in a 3:2 volume ratio, and stirred until the oil was fully dissolved. After adding 0.5 mL of saturated potassium iodide solution, the mixture was placed in a dark location for one minute. Subsequently, 30 mL of distilled water was added to the mixture, followed by titration with 0.01 N sodium thiosulfate. The titration process continued until the yellow color was removed, at which point 0.5 mL of 1% starch indicator was added. The

titration was continued until the disappearance of the blue color. Finally, the peroxide value of the oil was calculated as milliequivalents (mEq) of peroxide per kilogram of oil.

The iodine number was determined following the AOCS method No. Cd 1c-85 [19]. Approximately 0.5 grams of solid oil or 0.2 grams of liquid oil were weighed and dissolved in 10 mL of chloroform. To the solution, 25 mL of Hanus iodine solution was added slowly with gentle stirring, and the flask was kept in the dark for 30 minutes after placing the glass cork. The lid of the flask was then removed, and the inside was washed successively with 20 mL of potassium iodide solution and 100 mL of distilled water. The solution was titrated with sodium thiosulfate until a yellow color appeared. Subsequently, 2 mL of starch indicator was added, and titration was continued until the blue color disappeared.

To calculate the saponification number, the AOCS method number Cd 3-25 [20] was employed. Five grams of oil were mixed with 50 mL of a 0.5 N alcoholic potassium hydroxide solution, with potassium added slowly in 15 seconds. The mixture was heated for 30 minutes by connecting the flask to a condenser placed over a boiling water bath. After cooling, 1 mL phenolphthalein was added to the flask, and the excess potassium hydroxide was titrated with a 0.5 N HCl to determine the amount of non-participating soap present.

2.5. Determination of free fatty acid composition

To analyze the free fatty acid (FFA) composition in the oil, a gas chromatography-mass spectrometry (GC-MS) device was employed (Agilent 6890N). The oil sample was first methylated in order to be injected into the GC device. This was achieved by mixing 60 mg of oil with 3 mL of hexane and 0.3 mL of 2N methanolic potassium hydroxide and shaking the mixture for 25 minutes at room temperature using a LABO MX-F tube shaker. Subsequently, the sample was transferred to Eppendorf and subjected to centrifugation (Sigma 2-16P) at a speed of 15,000 rpm for 12 minutes. The clear layer on top was then removed and injected into the GC-MS device using a DB-WAX column, 30 m long column with hydrogen gas as the carrier gas at a flow rate of 1 mL/min. A flame ionization detector (FID) was used, and the injection was done in a divided volume of 1 L. The column temperature was raised from 180 to 240 ℃ at a rate of 4 ℃ per minute, while the detector and injector temperatures were both set at 250 ℃.

2.6. Measurement of Phenolic Content and Antioxidant Activity

To determine the levels of phenol and antioxidant activity, it is crucial to extract these compounds from the oil. Hence, 2.5 g of the oil was mixed with 2.5 mL of n-hexane and centrifuged for 5 min to extract the compounds. The resulting extract, obtained from the clear upper layer, was then utilized to measure the total phenol and antioxidant capacity through testing.

To measure the total phenolic compounds (TPC), the Folin–Ciocalteu method was employed. Initially, 0.4 g of dry gallic acid was dissolved in 10 mL of ethanol and made up to a volume of 100 mL with distilled water to prepare the main solution. Then, 100 mL volumetric flasks were filled with distilled water to draw the calibration curve. The flasks contained solutions with varying concentrations of gallic acid, which were 0, 25, 50, 100, 250, 500, and 1000 mg/L. In this experiment, 20 L of the extract were mixed with 1.16 mL of distilled water, 100 L of 10% Folin–Ciocalteu reagent, and 300 L of 20% calcium carbonate solution. The mixture was kept in a Bain-Marie at 40 ℃ for 3 minutes and the absorbance was measured at 760 nm using a Rayleigh UV-9200 UV/Vis spectrometer. Finally, the TPCs of the extract were calculated based on the standard curve of gallic acid (Figure 1) and expressed as equivalent milligrams of gallic acid per gram.

To measure the antioxidant property, the DPPH radical scavenging method was employed. In this method, 0.2 mL of the extract was combined with 4 mL of a 0.06 mM DPPH free radical methanolic solution. The resulting mixture was stirred and left in a dark place at room temperature for 60 minutes. The absorbance of the samples was measured at 517 nm using Rayleigh UV-9200 UV/Vis spectrometer. A control sample of 0.06 mM DPPH methanolic solution and methanol solvent were used.

2.7. Analysis of Sterol Profile

The sterol composition in the oils was assessed following the ISO-12228 (1999) methodology. The gas chromatography (GC) system employed was a Young Lin 6000, equipped with a flame ionization detector and a TYM5 capillary column of 30 m length, 0.25 mm internal diameter, and 0.25 μm film thickness. The column was subjected to a constant flow of hydrogen at a rate of 2 mL/min, with a split ratio of 40:1. The temperatures for both the injector and detector were set at 290 °C and 300 °C, respectively. The GC oven was operated

under isothermal conditions at 268 °C for a duration of 50 minutes. The final outcome for each sterol component was represented as a percentage concentration.

2.8. Assessment of Tocopherol Profile and Thymoquinone Content

The constitution of tocopherols (alpha, beta, gamma, and theta-tocopherols) was studied using an adapted technique from the AOCS (1997). Utilizing high-performance liquid chromatography (HPLC), tocopherols were measured via direct injection of BCO into a solvent blend of heptane and tetrahydrofuran (ratio 95:5, v/v). The detection and quantification process was executed using a Young Lin SD 30 Plus, and a UV-500/GTI detector with wavelengths configured at 295 nm for excitation and 330 nm for emission. The column employed was a LICHROSPHER 25 cm × 4.6 mm i.d., filled with Supelcosil Luna, 5 µm. The mobile phase incorporated a mixture of heptane and tetrahydrofuran (95:5, v/v) with a flow velocity of 1.2 mL/min, and an injection volume of 10 µL. For the identification and quantification of peaks, standards of alpha, beta, gamma, and theta isomers of tocopherols were dissolved in hexane. The tocopherol content within the oils was calculated in terms of mg of tocopherols per 100g of oil, based on external calibration curves (r = 0.999), established separately for each tocopherol standard.

The method employed for the analysis of thymoquinone content in Nigella sativa oil was adapted from the work previously published [21]. Nigella sativa oil samples were prepared by adding 1 mL of methanol to 1 mL of oil in a glass centrifuge tube. The mixture was vortexed for 2 minutes, after which the methanol top layer was transferred to a glass tube. The methanol was then evaporated under a nitrogen stream, and the residue was reconstituted with 1 mL of mobile phase [21] then the HPLC system used for the analysis. The mobile phase consisted of hexane:2 propanol (99:1 v/v), and thymoquinone was monitored by UV detection at 295 nm. This method was found to be quite specific and sufficiently sensitive, with a lower limit of 5 nmoles/mL. This method provides a simple, reliable, and sensitive approach for the routine analysis of thymoquinone in Nigella sativa oil, which is crucial for ensuring the quality and efficacy of the final produced oil.

3. Results and Discussion

3.1. Physicochemical Properties of Raw Black Cumin Seeds and Oil Yield

The raw BCS used in this study had a moisture content of 3.25±0.28%, an oil content of 37.53±0.73%, a protein content of 18.71±0.48%, and an ash content of 5.16±0.47%. These values are within the typical range for BCS, indicating that the seeds were of good quality. The oil yield obtained through the cold press extraction method was 22.33±0.58%, which is a reasonable yield considering the oil content of the seeds and the extraction method used.

3.2. Physicochemical Properties of Cold Press Extracted Nigella Sativa Oil

The extraction of oil from Nigella Sativa seeds using the cold press method yielded an oil content of 22.33±0.58%. This extraction method is known for its efficiency and the high quality of oil it produces, as it does not involve the use of heat or solvents that could degrade the oil's quality.

The physicochemical properties of the oil were analyzed, and tabulated in Table 1, which revealing a Free Fatty Acid (FFA) level of 8.24%, a peroxide value (PV) of 9.56 meq O2/kg oil, an iodine value of 119.27 I2/100 g, and a saponification number of 204.76 mg of KOH/g of oil. These values are within the expected ranges for Nigella Sativa oil, indicating that the cold press extraction method was successful in preserving the oil's quality.

The FFA level is a measure of the oil's acidity, and a lower value is generally preferred as it indicates a lower degree of hydrolysis. The PV, on the other hand, is a measure of the extent of lipid oxidation, with a lower value indicating a lower degree of oxidation and thus a higher quality of oil. The iodine value indicates the degree of unsaturation in the oil, with a higher value indicating a higher degree of unsaturation and thus a higher potential for oxidation. The saponification number is a measure of the oil's average molecular weight or chain length, with a higher value indicating a higher proportion of shorter-chain fatty acids.

3.3 Impact of Cold Press Extraction on Fatty Acid Composition and Bioactive Lipids

The fatty acid composition of the Nigella Sativa oil was analyzed using Gas Chromatography (GC) with a flame ionization detector (FID). Figure 1 shows the gas chromatogram of Nigella sativa oil, revealing a high concentration of unsaturated fatty acids. Based on the GC

spectroscopy data of Nigella sativa oil extracted by cold press, the mean concentrations and standard deviations of the identified free fatty acids (FFAs) are calculated.

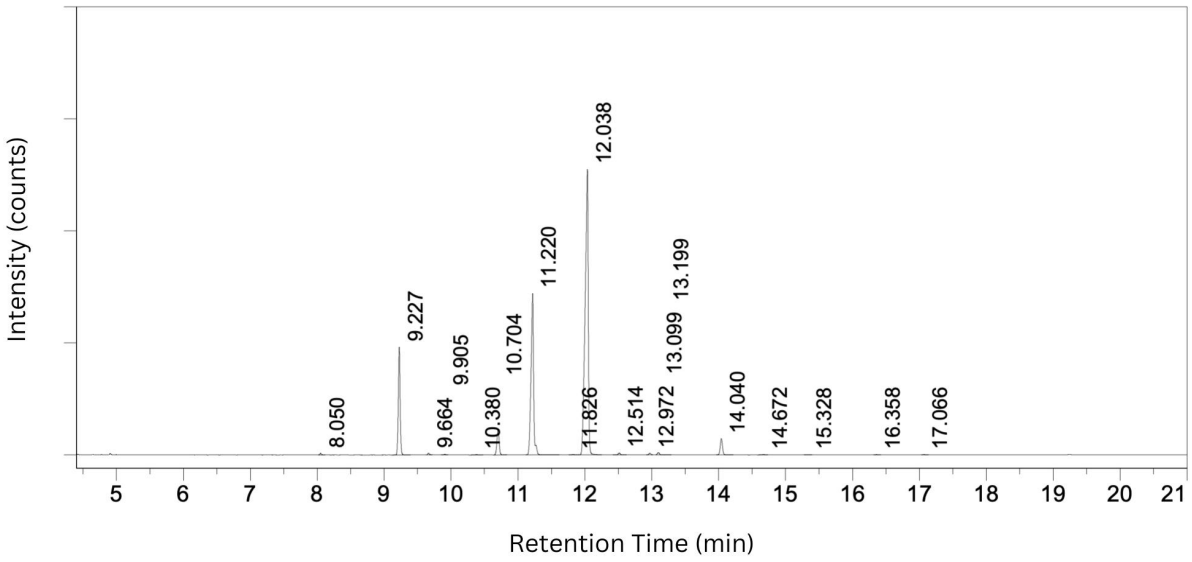

Figure 1. Gas chromatogram of Nigella sativa extracted oil

The results are summarized in Table 1. The most abundant fatty acid in the oil was linoleic acid (C18:2c), accounting for approximately 55.06% of the total fatty acids, followed by oleic acid (C18:1c) and palmitic acid (C16:0) at 25.02% and 12.33%, respectively. The high concentration of linoleic acid, an essential omega-6 fatty acid, is consistent with previous studies on Nigella sativa oil, reinforcing its potential nutritional and health benefits [22-24].

Table 1. Summarized free fatty acid analysis along with corresponding acid name

FFA	**Corresponding Acid Name**	**Concentration**
C14:0	Myristic acid	0.173 ± 0.006
C16:0	Palmitic acid	12.334 ± 0.364
C16:1	Palmitoleic acid	0.2085 ± 0.0196
C17:0	Margaric acid	0.065 ± 0.01
C17:1	Heptadecenoic acid	0.0565 ± 0.0181
C18:0	Stearic acid	3.43025 ± 0.2206

C18:1c	Oleic acid	25.01875 ± 1.1159
C18:2t	trans-Linoleic acid	0.0813 ± 0.0335
C18:2c	Linoleic acid	55.05925 ± 0.4027
C18:3c	α-Linolenic acid	1.105 ± 1.2374
C18:3n3	γ-Linolenic acid	0.2295 ± 0.0007
C20:0	Arachidic acid	0.22275 ± 0.0289
C20:1	Eicosenoic acid	0.321 ± 0.0194
C22:0	Behenic acid	0.04425 ± 0.0184
C22:2	Docosadienoic acid	0.0395 ± 0.0007
C22:1	Erucic acid	0.0305 ± 0.0007
C24:0	Lignoceric acid	0.03175 ± 0.0089
C20:2	Eicosadienoic acid	2.24875 ± 0.0392
Others	-	0.082 ± 0.0401

The presence of α-linolenic acid (C18:3c) and γ-linolenic acid (C18:3n3), both omega-3 fatty acids, albeit in lower concentrations, further enhances the nutritional value of the oil.

Also, these FFAs, particularly linoleic acid and oleic acid, contribute to the nutritional and therapeutic properties of Nigella sativa oil. Linoleic acid, an essential omega-6 fatty acid, and oleic acid, a monounsaturated omega-9 fatty acid, are known for their beneficial effects on heart health [25, 26].

The p-value analysis indicates that the differences in the concentrations of the FFAs are statistically significant. This suggests that the cold press extraction method and possibly other factors such as the seed's origin, maturity, and storage conditions could influence the FFA profile of Nigella sativa oil.

In conclusion, the GC spectroscopy data provides valuable insights into the FFA composition of Nigella sativa oil. Further studies are needed to explore the impact of various factors on the FFA profile and to elucidate the correlation between the FFA composition and the oil's nutritional and therapeutic

3.4. Impact of Cold Press Extraction on Sterol Composition

The sterol composition of the cold-pressed Nigella sativa oil was also analyzed. Sterols, also known as phytosterols, are naturally occurring compounds found in plant cell membranes. They are structurally similar to cholesterol and have been associated with various health benefits, including cholesterol-lowering effects. The results of the sterol analysis are presented in Table 2.

Table 2: Sterol profile composition of cold-pressed Nigella sativa oil

Sterol	**Percentage (%)**
Cholesterol	0.18
Campesterol	11.25
Stigmasterol	11.97
Beta-sitosterol	53.74
Sitostanol	2.09
Delta-5-avenasterol	15.54
Delta-5,24-stigmastadienol	1.12
Delta-7-stigmastenol	1.96
Delta-7-avenasterol	2.01
Other	0.14

The most abundant sterol in the oil was beta-sitosterol, accounting for approximately 53.74% of the total sterol content. This was followed by delta-5-avenasterol and campesterol, contributing 15.54% and 11.25% of the total sterol content, respectively. The total sterol content of the oil was determined to be 2209.78 ppm.

The high content of beta-sitosterol, a plant sterol known for its cholesterol-lowering properties, is noteworthy. This finding is consistent with previous studies on Nigella sativa oil and reinforces its potential health benefits.

In comparison with the literature, the sterol content of our sample is in line with previously reported values for cold-pressed Nigella sativa oil. However, variations in sterol content can occur due to differences in growing conditions, extraction methods, and seed variety.

In conclusion, the high sterol content of cold-pressed Nigella sativa oil, particularly beta-sitosterol, further enhances its nutritional value and potential health benefits. Further studies are needed to explore the health benefits and potential applications of this oil in the food and pharmaceutical industries.

3.5. Tocopherol Composition Analysis of Cold-Pressed Nigella Sativa Oil

The tocopherol composition of the cold-pressed Nigella sativa oil was further analyzed and quantified. Tocopherols, a group of fat-soluble alcohols with Vitamin E activity, are known for their antioxidant properties. The results of the tocopherol analysis are presented in Table 3.

Table 3: Tocopherol composition of cold-pressed Nigella sativa oil

Tocopherol	**Concentration (mg/100g)**
Alpha-Tocopherol	4.57
Beta-Tocopherol	2.83
Gamma-Tocopherol	5.58
Delta-Tocopherol	2.17
Total	15.15

The most abundant tocopherol in the oil was gamma-tocopherol, followed by alpha-tocopherol, beta-tocopherol, and delta-tocopherol. The total tocopherol content was determined to be 15.15 mg/100g.

The chromatogram of alpha-tocopherol is presented in Figure 2. The alpha-tocopherol peak was identified at a retention time (RT) of 5.0167 min, with an area of 3656.6948 mV.s, a height of 323.9948 mV, and accounted for 21.27% of the total area. Two other significant

peaks were observed at RTs of 6.4833 min and 6.8833 min, representing 61.61% and 17.12% of the total area, respectively. The total area under the curve was 17195.7852 mV.s.

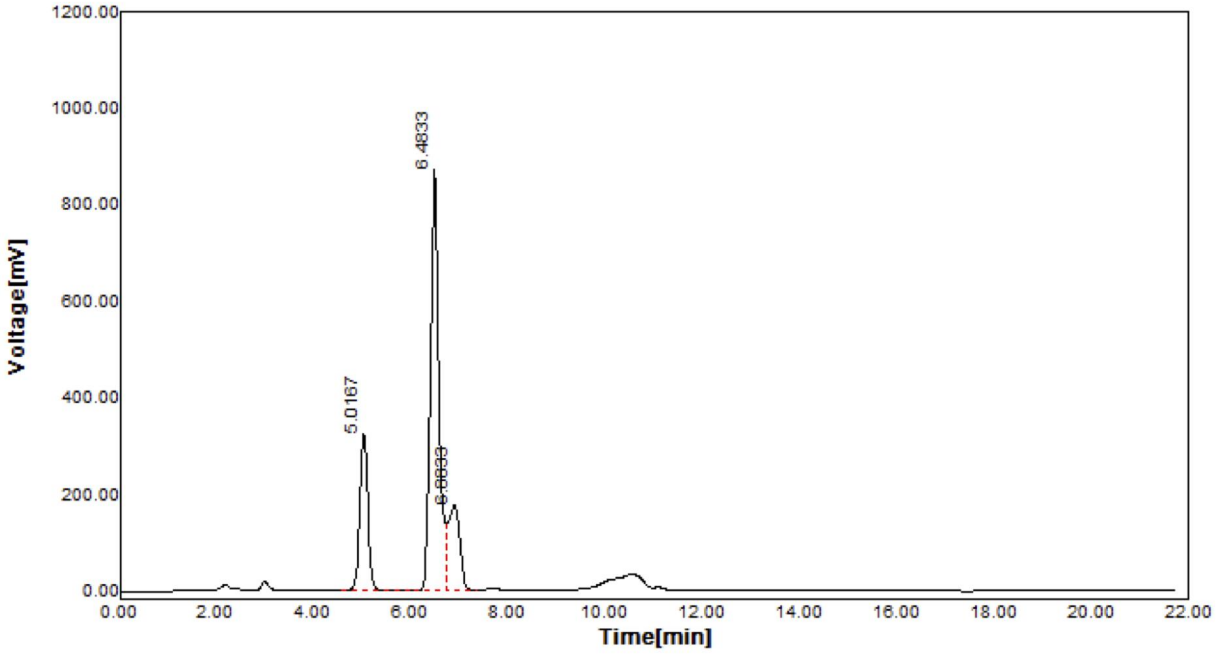

Figure 2. Chromatogram of Tocopherol content in cold press extracted Nigella sativa oil

The high content of gamma-tocopherol and alpha-tocopherol in the oil is noteworthy. These tocopherols are known for their potent antioxidant properties, which can protect the oil from oxidative degradation, thereby enhancing its shelf life. Furthermore, dietary tocopherols have been associated with various health benefits, including reduced risk of chronic diseases such as heart disease and cancer.

In comparison with the literature, the tocopherol content of our sample is consistent with previously reported values for cold-pressed Nigella sativa oil. However, variations in tocopherol content can occur due to differences in growing conditions, extraction methods, and seed variety.

In conclusion, the high tocopherol content of cold-pressed Nigella sativa oil, particularly gamma-tocopherol and alpha-tocopherol, further enhances its nutritional value and potential health benefits. Further studies are needed to explore the health benefits and potential applications of this oil in the food and pharmaceutical industries.

3.6. Total Phenolic Content and Antioxidant Potential

The total phenolic content (TPC) of the oil was 114.5 mg Gallic acid/kg oil. Phenolic compounds are known for their antioxidant properties, and the TPC is a measure of the oil's potential antioxidant activity. The rate of free radical inhibition in the cold-pressed oil was found to be 40.74%, indicating a strong antioxidant potential. This is consistent with the known antioxidant properties of Nigella Sativa oil, and suggests that the cold press extraction method was effective in preserving these properties.

3.7. Thymoquinone Saturated Nigella Sativa Oil

In alignment with the research objective of enhancing the thymoquinone concentration in Nigella sativa oil for nutritional purposes, our analysis revealed a noteworthy presence of this bioactive compound. Thymoquinone, recognized for its potent antioxidant and anti-inflammatory properties, was found to constitute 0.28% of the cold-pressed Nigella sativa oil. This concentration, while significant, was lower than the 60% thymoquinone content observed in the extracted essence.

To augment the thymoquinone concentration in the oil, a saturation process was employed using the extracted essence. This procedure resulted in a substantial increase in the thymoquinone content, raising it to 2.96%. This marked increase underscores the efficacy of the cold press extraction method in preserving the intrinsic thymoquinone content of Nigella sativa seeds, and demonstrates the potential of the saturation process as a viable strategy for further enhancing this content. This finding is particularly significant given the potential health benefits associated with thymoquinone, and suggests promising avenues for the development of nutritionally enriched Nigella sativa oil products.

The results of this study are consistent with previous research on Nigella Sativa oil. For instance, a study by Ramadan and Mörsel [27] reported similar findings regarding the fatty acid composition of the oil, with linoleic and oleic acids being the major unsaturated fatty acids, and palmitic acid being the major saturated fatty acid. The high content of unsaturated fatty acids in Nigella Sativa oil has been associated with various health benefits, including a reduced risk of heart disease and improved blood lipid profiles.

The thymoquinone content of the oil in this study (0.28%) is lower than the values reported in some previous studies. For example, a study by Lutterodt *et al.* [5] reported a thymoquinone content of up to 8.73 mg/g of oil. However, it should be noted that the thymoquinone content

can vary depending on the extraction method and the source of the seeds. In this study, the thymoquinone content was significantly increased (to 2.96%) by saturating the oil with the extracted essence, suggesting a potential method for enhancing the thymoquinone content of the oil.

The total phenolic content and antioxidant potential of the oil in this study are also consistent with previous research. A study by Ramadan *et al.* [28] reported a total phenolic content of 245 mg gallic acid/kg oil and a free radical inhibition rate of 78.4% for cold-pressed Nigella Sativa oil. These values are higher than those found in this study, which could be due to differences in the extraction method or the source of the seeds.

In conclusion, this study has demonstrated that the cold press extraction method is effective in extracting oil from Nigella Sativa seeds, preserving its quality and bioactive properties, and enhancing its thymoquinone content. The oil has a high content of unsaturated fatty acids, a significant amount of thymoquinone, and a strong antioxidant potential, making it a valuable source of dietary lipids with potential health benefits. Further research is needed to explore the potential applications of this oil in food, cosmetics, and pharmaceuticals, and to investigate methods for further enhancing its bioactive properties.

4. Conclusion

The present study provides a comprehensive analysis of the physicochemical properties, fatty acid composition, and antioxidant potential of cold-pressed Nigella Sativa oil enriched with thymoquinone. The results revealed that the oil is rich in unsaturated fatty acids, particularly linoleic and oleic acids, and has a thymoquinone content of 0.28%. The enrichment process significantly increased the thymoquinone content to 2.96%, enhancing the oil's antioxidant potential. The cold-pressed extraction method preserved the oil's quality, as indicated by its good physicochemical properties, including a reasonable free fatty acid content, peroxide value, iodine value, and saponification number. The oil also exhibited a high content of sterols, particularly beta-sitosterol, and tocopherols, which are known for their health benefits. The findings of this study suggest that cold-pressed Nigella Sativa oil enriched with thymoquinone has promising potential as a functional ingredient in the food and pharmaceutical industries due to its good physicochemical properties and high antioxidant potential. However, further research is needed to explore the potential health benefits of this enriched oil and to optimize the enrichment process for commercial applications.

In conclusion, this study contributes to the growing body of research on Nigella Sativa oil and its potential applications in the food and pharmaceutical industries. The enrichment of the oil with thymoquinone, a compound known for its antioxidant and anti-inflammatory properties, could enhance its health benefits and broaden its applications. Future studies should focus on exploring these potential benefits and applications, as well as optimizing the enrichment process for commercial use.

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