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Oxidative Stability and Bioactive Composition of Marketed *Nigella sativa* L. Oils: A Comparative Evaluation

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Abstract

In this study, the physicochemical properties and oxidative stability of 10 commercial (A–K) and one laboratory-produced (M) cold-pressed black cumin (*Nigella sativa* L.) oils were evaluated. Analyses included peroxide value, free fatty acidity, fatty acid composition, and thymoquinone (TQ) content. Among the samples, sample C had the highest peroxide value (88.57 meq O_2/kg) and thymoquinone concentration (19.79 mg/g oil), whereas the highest free fatty acidity was observed in sample K (18.53%). Oxidative stability was assessed using the Rancimat method and an accelerated oven storage test at 65 °C over 30 days. During storage, peroxide values ranged from 9.84 to 124.54 meq O_2/kg, showing both increases and decreases depending on the sample. Induction times determined by Rancimat varied between 1.76 and 15.82 hours, indicating an approximately eightfold difference in oxidative resistance among oils. Fatty acid analysis revealed that the oils were rich in linoleic acid (48.30–57.85%) and oleic acid (25.69–42.10%), with palmitic acid ranging from 5.32 to 10.91%. Significant variations in TQ content (0.11–19.79 mg/g oil) were detected, which may be attributed to factors such as seed origin, harvest time, extraction methods, and potential adulteration. These findings demonstrate that the quality of cold-pressed black cumin oils on the market can vary substantially, with some products already exceeding international peroxide and FFA limits or showing unusually low TQ content. This variability highlights the need for standardized production practices and regulatory quality criteria to ensure product safety, authenticity, and consistency.

Keywords: Black cumin, Fatty acid, Induction time, Peroxide, Thymoquinone.

1. Introduction

Today, changing lifestyles and dietary habits have significantly increased the demand for natural health-promoting solutions, drawing growing attention to plant-based products. Consequently, interest in products derived from natural raw materials has risen, encouraging manufacturers to develop functional and health-oriented alternatives. Among such products,

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vegetable oils have gained popularity not only in the food sector but also in cosmetics and pharmaceuticals due to their diverse health benefits and increasing market demand.

Vegetable oils exhibit antioxidant, anti-inflammatory, antimicrobial, and immune-supporting properties thanks to their biologically active compounds, such as essential fatty acids, phenolics, tocopherols, and phytosterols (Kaur & Saraf, 2010; Ramadan, 2004). As a result, their application in functional foods is expanding globally, including in Türkiye (Hashemi et al., 2021). Among these oils, black cumin (*Nigella sativa* L.) oil stands out due to its potent bioactive constituents, particularly thymoquinone, which is known for its immunomodulatory, hepatoprotective, anticancer, and antioxidant effects (Ahmad et al., 2019; Gholamnezhad et al., 2016). Despite its therapeutic potential, there is currently no specific quality standard for black cumin oil in the Turkish Food Codex Communiqué on Oils Named by Plants (2012/29). Consequently, there is an urgent need for quality-based regulations and standardized analytical methods at both national and international levels to ensure product reliability and consumer safety.

The black cumin plant (*Nigella sativa* L.), an annual herb cultivated across the Eastern Mediterranean, Southern Europe, India, and Egypt, is also widely grown in Thrace, Northern Anatolia, and the Mediterranean regions of Türkiye (Tonçer & Kızıl, 2004; Baytöre, 2011). Its seeds are rich in fixed oil (35–41%), protein (22%), dietary fiber, vitamins (β-carotene, tocopherols, folic acid), minerals, and various phytochemicals (Ramadan & Mörsel, 2004; Kiralan et al., 2014). However, oil composition can vary depending on genotype, climate, harvest time, processing, and storage conditions (Cheikh-Rouhou et al., 2007). Thymoquinone, the main pharmacologically active compound, is often not declared on product labels, and its concentration varies substantially based on genetic background and production techniques (Aboutabl et al., 1986; Farag et al., 2017; Alkhatib et al., 2020). Cold-pressed oil is gaining more attention and demand because it does not require refining and is free from solvents and chemicals. Cold-pressed black cumin oil contains many phenolic and health-enhancing bioactive components; the most important of these is thymoquinone (Alkhatib et al., 2020).

The cold press method is a solvent-free extraction technique that retains the oil's natural structure and preserves heat-sensitive bioactive compounds, including thymoquinone and antioxidants (Lutterodt et al., 2011; Gecgel et al., 2016). It is often preferred for the production of high-quality black cumin oil, especially when processing temperatures remain below 50 °C. One of the main challenges encountered in black cumin oil production is that the peroxide (PV) and acidity (AV) values can be relatively high even on the day the oil is harvested (Ashrafi et

al., 2023). Different approaches have been developed to reduce these parameters (Agah et al., 2014).In recent years, although there has been a growing number of studies on the chemical composition and pharmacological properties of Nigella sativa oil, comparative investigations focusing on the oxidative stability and bioactive composition of commercially available products remain very limited (Szydłowska-Czerniak et al., 2022; Hashemi et al., 2022). Most previous works have either concentrated on a single extraction method (Kiralan et al., 2014), a narrow set of physicochemical parameters (Argon & Gökyer, 2016), or non-commercial seed sources (Hamrouni-Sellami et al., 2008), which makes it difficult to draw conclusions about the real variability consumers face in the marketplace. Furthermore, there is a lack of systematic data linking peroxide value, free fatty acidity, induction time, and thymoquinone concentration together as integrated quality indicators. This gap prevents the establishment of reference ranges that could guide both consumers and regulatory authorities (Cheikh-Rouhou et al., 2007; Khaikin et al., 2022). Therefore, studies that evaluate multiple quality criteria in parallel on a wide range of commercial oils are essential to highlight potential risks of adulteration, mislabeling, or poor storage conditions (Oubannin et al., 2022).

In this context, the aim of this study is to comparatively evaluate the quality characteristics of commercially available cold-pressed black cumin (Nigella sativa L.) oils, and to reveal their thymoquinone content and oxidative stability using a holistic approach. By considering multiple parameters such as peroxide value, free fatty acid, Rancimat induction time, and fatty acid profile together, the study aims to quantitatively determine the true quality variation among commercial products and to emphasize the need for standardized quality criteria at national and international levels. (Codex Alimentarius Commission, 1999; Resmî Gazete, 2017).

2. MATERIALS AND METHOD

Material

Ten cold-pressed black cumin (*Nigella sativa* L.) oil samples were obtained from the domestic market (Table S1). Black cumin seeds were sourced from a local producer. The seeds were dried on blotting paper at room temperature, away from direct sunlight, to remove excess moisture. Once dried, the samples were homogenized using a mechanical grinder and prepared for analysis. Additionally, as a control sample, one thousand grams of black cumin seeds were subjected to mechanical pressing under controlled conditions, ensuring the processing temperature did not exceed 45°C. After pressing, the crushed seed material was stored under refrigerated conditions for three nights to facilitate the separation of the oil from the fibrous

matrix. Subsequently, the oil phase was collected and clarified by filtration using a syringe filter tip (adapted from Muhialdin et al., 2016). The resulting black cumin oil, used as a control sample, was stored in the dark at 4 °C until further analysis.

2.2. Method

2.2.1. Peroxide value

The peroxide value was determined using the AOCS Official Method Cd8-53 (AOCS, 1990) with slight procedural consistency. For the analysis, about 2–5 g of oil was weighed, after which 10 mL of chloroform and 15 mL of acetic acid were added to the sample to obtain a homogeneous mixture. Then, 1 mL of saturated potassium iodide was introduced, the container was sealed immediately, gently agitated for one minute, and kept in the dark for a five-minute reaction period. Following this step, 75 mL of distilled water was added along with 4–5 drops of starch solution as an indicator. The resulting mixture was titrated with 0.002 N sodium thiosulfate until the disappearance of the blue color. The volume of titrant used was noted, and the peroxide value (meq O_2/kg) was subsequently calculated using the standard formula.

Peroxide value = (V/m) × 2.8

V: Volume of sodium thiosulfate used in titration (mL)

m: Mass of the oil sample (g)

2.2.2 Free fatty acidity

Free fatty acidity was quantified in accordance with the AOAC Official Method described by Latimer (2012). Approximately 2 g of oil was transferred into an Erlenmeyer flask and mixed with 75 mL of a 1:1 (v/v) blend of 95% ethanol and diethyl ether. After homogenization, 3–4 drops of phenolphthalein indicator were added. The mixture was then titrated with 0.1 N ethanolic potassium hydroxide until a persistent pale pink color signaled the endpoint. The free fatty acidity, expressed as oleic acid percentage, was subsequently computed using the following formula:

Free fatty acidity (%) = (V / (m × 0.028)) × 100

V: Volume of KOH solution used in titration (mL)

m: Mass of the oil sample (g)

2.2.3. Induction time

The Rancimat method is an accelerated analysis technique used to evaluate the oxidation resistance of oils and oil-containing products. In this method, samples are subjected to oxidation

at a constant temperature and under a continuous airflow; the resulting volatile oxidation products are transferred to ultrapure water in a measuring cell, and the change in the electrical conductivity of the solution is monitored. This increase in conductivity reflects the progress of the oxidation process.

Oxidative stability was determined using the Rancimat method according to the AOCS Cd 12b-92 method; 3 g of oil sample was subjected to oxidation at 110 °C and under a constant airflow of 20 L/h, the increase in conductivity was monitored by transferring the resulting volatile compounds to 60 mL of ultrapure water, and the induction time was recorded in hours using a Rancimat 892 instrument (Metrohm AG, Herisau, Switzerland) (Anwar et al., 2013).

2.2.4. Oven Test (Schaal Oven Test)

The accelerated oven test was employed to estimate oxidative shelf life (Fennema, 1976). Samples were stored at 65 °C, and peroxide values were monitored at regular intervals (day 5, 10, 15, 20, 25, and 30). The temperature of 65 °C was chosen because it is commonly used in accelerated oxidation studies to mimic long-term storage under ambient conditions (Cuvelier & Maillard, 2012; Oubannin et al., 2022). Although this temperature is higher than that of normal storage environments, it enables the detection of oxidative differences between oils within a shorter experimental period.

2.2.5. Fatty acid composition (GS-MS)

To determine the fatty acid profile, 0.1 g of fat sample was weighed into 5 mL screw-on glass tubes. 0.2 mL of 2 N methanol-containing potassium hydroxide solution and a few drops of methyl orange as an indicator were added to the samples, and then the tubes were tightly sealed with PTFE septum caps. The tubes were shaken vigorously for approximately 30 seconds and allowed to stand until phase separation occurred (Parry et al., 2005). 1 µL of the resulting fatty acid methyl esters was taken and injected into a GC-MS (Gas Chromatography-Mass Spectrometry) system, and analyses were performed under these conditions.

GC–MSConditions

The analyses were performed on an Agilent 7890A gas chromatograph equipped with an Agilent 7683 autosampler. Separation was achieved using an HP-88 capillary column (100 m × 0.25 mm × 0.20 µm; p/n 112-88A7). The inlet temperature was maintained at 260 °C and injections were carried out in split mode with a 30:1 ratio. Helium served as the carrier gas under constant-flow conditions, providing a linear velocity of 20 cm/s. The oven program began

at 140 °C with a 5-minute hold, followed by heating at a rate of 4°C/min until reaching 240 °C, where the temperature was held for an additional 15 minutes. A 1-µL sample volume was injected for each analysis. Detection was performed using a 5975C mass selective detector, with the transfer line set to 280 °C. The solvent peak appeared at approximately 10.5 minutes, and data acquisition was conducted in scanning mode across the 40–400 amu mass range.

2.2.6. Thymoquinone analysis

For thymoquinone determination, 50 mg of each black cumin oil sample was mixed with 5 µL of methanol, vortexed, and centrifuged at 4000 rpm for 5 min at room temperature. The upper methanol phase was collected. The extraction step was repeated with an additional 5 µL of methanol, and both supernatants were combined and filtered through a 0.45 µm syringe filter. Standard thymoquinone solutions (0.0001–1000 ppm) were prepared in methanol and used to construct a calibration curve. Sample extraction was performed according to Ghosheh et al. (1999), using methanol-assisted phase separation. A calibration curve for thymoquinone was prepared in the range of 0.0001–1000 ppm, and a linear relationship was obtained ($R^2 > 0.995$). The limit of detection (LOD) and limit of quantification (LOQ) of the method were determined as LOD 0.078 ppm and LOQ 0.235 ppm, respectively.

Chromatographic Conditions

Mobile phase: 60% acetonitrile / 40% ultrapure water (isocratic)

Flow rate: 1.0 mL min^{-1}

Injection volume: 20 µL

Column: C18 (250 mm × 4.6 mm, 5 µm)

Column temperature: ambient

Detector: DAD at 254 nm

Thymoquinone was identified and quantified by comparing sample peak areas and retention times with those of the standard curve.

2.2.7. Statistical analyses

The data obtained from the oxidative stability and compositional analyses were evaluated using one-way analysis of variance (ANOVA) with the Minitab software (version 2000) (Tables S2-S4). Differences between means were determined using Tukey's multiple comparison test, and statistical significance was accepted at $P< 0.05$.

3. RESULTS AND DISCUSSION

3.1. Peroxide value

The peroxide, free fatty acid, induction period and thymoquinone values of cumin oil samples are shown in **Table 1**. The peroxide values of the black cumin oil samples analyzed in this study ranged from 9.84 to 88.57 meq O_2/kg, indicating considerable variation in oxidative status among the commercial products. The highest peroxide value was observed in sample C (88.57 meq O_2/kg), while the lowest value was detected in sample H (9.84 meq O_2/kg). The control sample (M), which was cold-pressed under laboratory conditions, exhibited a peroxide value of 29.85 meq O_2/kg. Among the commercial samples, B, E, H, and K displayed lower peroxide values than the control, suggesting relatively better oxidative quality at the time of analysis. According to Codex Stan 19-1981 Rev.2, peroxide values for virgin and cold-pressed oils should not exceed 15 meq O_2/kg (Codex Alimentarius Commission, 1999). Our results indicate that only samples H (9.84) and B (13.70 meq/kg) conform to this international benchmark. This finding clearly indicates that most of the commercial oils tested in this study do not comply with international benchmarks and may already be partially oxidized at the time of purchase. From a consumer perspective, this raises concerns about both safety and shelf life. Furthermore, ISO 3960:2007 specifies that peroxide determination methods are reliable up to approximately 30 meq O_2/kg (International Organization for Standardization, 2007). Although the Turkish Food Codex does not currently define explicit peroxide limits for black cumin oil, analogous regulations for margarine and similar fat spreads set a maximum peroxide value of 5 meq O_2/kg under TSE TS 2812 (TSE, 2018). Meanwhile, the Turkish Food Codex (2017/26) for olive oils references international analytical methods (e.g., TS EN ISO 3960). Comparative studies from the literature report peroxide values of 25.9 meq/kg (Argon & Gökyer, 2016) and 31.3 meq/kg via cold press, with lower values for soxhlet (25.2 meq) and microwave extraction (21.5 meq) methods (Kiralan et al., 2014). In the study conducted by Agah et al. (2024), it was reported that microwave pretreatment of black cumin seeds for 0–2.5 minutes before cold pressing increased the extraction yield of the oil obtained; however, it significantly reduced the acid and peroxide values..The even higher values observed here (up to 88.57 meq O_2/kg) may reflect inadequate storage, delayed seed processing, or adulteration practices, which are rarely reported in product labels. These findings fall within or slightly exceed the ISO applicability range. The large discrepancies in peroxide values among samples likely derive not only from seed origin and genotype but also from inadequate storage, adulteration, and poor packaging practices, which may explain the extremely high levels observed in some commercial oils.

Exceeding recommended peroxide levels signifies the presence of primary oxidation products which can adversely affect both sensory quality and shelf life. Consequently, adherence to standard production protocols and post-production monitoring is essential for improving the oxidative quality of cold-pressed black cumin oil.

3.2. Free Fatty Acidity (FFA)

The free fatty acidity (FFA) of the black cumin oil samples varied considerably, ranging from 0.00% to 18.53% (expressed as % oleic acid) (**Table 1**). The highest FFA value was recorded in sample K (18.53%), while the lowest was detected in sample F (0.00%). The laboratory-produced control oil (sample M) exhibited a free fatty acidity of 4.57%, which was exceeded by several commercial samples, including A, D, E, and K, indicating potential hydrolytic degradation. Free fatty acids are formed primarily through the hydrolysis of triacylglycerols, a process that is accelerated under poor processing and storage conditions such as exposure to high temperature, moisture, air, and light. High FFA levels are generally considered undesirable, as they may negatively impact oxidative stability, organoleptic quality, and shelf life (Dong et al., 2020). According to Codex Alimentarius Standard 210, the maximum acid value permitted for cold-pressed edible oils is 4 mg KOH/g oil, corresponding approximately to 2% free fatty acids expressed as oleic acid. However, stricter limits have been reported in some national standards, such as INSO Standard 13392, which specifies a maximum free fatty acid level of 1.5% (as oleic acid). Therefore, most samples in this study, excluding samples B, C, F, G, H, I, and M, exceed international recommendations, raising concerns about their quality control, storage, or potential adulteration. These findings are consistent with previous studies. For instance, Choudhury et al. (2023) reported an FFA value of 6.66% for cold-pressed black cumin oil, while Sultan et al. (2009) observed a wide FFA range between 4.7% and 20.5%, depending on variety and origin. Similarly, Aftab et al. (2014) noted FFA levels of 22.7% and 18.6% in oils extracted with hexane from seeds cultivated in Tunisia and Iran, respectively. The high FFA values in some commercial samples in our study indicate that these oils may already be degraded or unsuitable for human consumption according to international standards, emphasizing the importance of stricter regulation and shelf-life control.

3.3. Induction Time (Oxidative Stability)

The stability period of oils against oxidative deterioration is expressed as the induction period. The longer this period is, the higher the oxidative stability of the oil. (Czerniak et al., 2022). The induction times of the black cumin oil samples, determined by the Rancimat method, varied

significantly between 1.76 and 15.82 hours, indicating substantial differences in oxidative stability **(Table 1)**. The highest induction time was observed in sample F (15.82 h), suggesting strong oxidation resistance, whereas the lowest was recorded in sample K (1.76 h), indicating high susceptibility to rancidity. The control sample (M) demonstrated moderate oxidative stability with an induction time of 9.22 hours. Oxidative stability is one of the most critical parameters reflecting the shelf life and quality of vegetable oils, as it is affected by various factors including fatty acid composition, antioxidant content, processing method, and storage conditions. A longer induction time generally indicates better protection against oxidative deterioration (Gorjanović et al., 2011). Previous studies have reported induction periods ranging from 3 to 14 hours for Nigella sativa oils, depending on production conditions and antioxidant concentration (Cheikh-Rouhou et al., 2007; Gecgel et al., 2016). Kiralan et al. (2014) reported in their study that the induction times of Soxhlet and microwave-assisted extracted black cumin oils were 19.6 hours and 18.4 hours, and the induction time of cold-pressed black cumin oil was 3.48 hours. It may be that cold-pressed oil contains higher amounts of antioxidant compounds compared to solvent-extracted and microwave-assisted extracted oils (Sookwongh et al., 2024). The remarkable variability in this study highlights that commercially available black cumin oils may exhibit up to nine-fold differences in oxidative resistance, underscoring the need for standardized production and storage protocols to ensure product quality.

3.4. Thymoquinone Content

The thymoquinone concentrations of the black cumin oil samples are presented in **Table 1** and **Figure 1**. Thymoquinone is one of the principal bioactive compounds in Nigella sativa oil, known for its anticancer, antimicrobial, and anti-inflammatory properties (Ahmad et al., 2019). In this study, the thymoquinone contents of the samples differed significantly ($p < 0.05$), ranging from 0.11 to 19.76 mg/g oil. The highest level was detected in sample C, whereas the control sample (M), produced fresh under cold-pressing conditions in the laboratory, contained 4.75 mg/g oil. Samples A and G showed the closest values to the control, while the lowest thymoquinone concentrations were found in samples F, H, and B.

The variability in thymoquinone content can be attributed to several factors including seed origin, maturity stage, environmental conditions, and extraction method (Telci et al., 2021). Sakdasri et al. (2023) highlighted that seed feeding rate, temperature, and moisture content influence both oil yield and thymoquinone levels. Similarly, Khaikin et al. (2022) reported values ranging from 0.45 to 8.09 mg/g oil among five different commercial black cumin oil

brands. In addition, Telci et al. (2021) noted thymoquinone contents of 2.3 and 1.9 mg/100 mg in the first and second harvest years of N. sativa grown under Mardin's climatic conditions. Abedinzadeh et al. (2024) reported significant differences in quality parameters between black cumin oils obtained by hot pressing and oils obtained from the post-press pulp using a solvent. In particular, the extraction method was decisive in determining the peroxide value, acid value, and bioactive component profile. The wide range of PV and thymoquinone observed among commercial samples in our study suggests that products on the market may have different extraction histories. Abedinzadeh et al. (2023) reported that the refining process significantly reduced the thymoquinone content in black cumin oil, and that the highest thymoquinone level was found in unrefined cold-pressed oil. This situation may explain the extremely low levels of thymoquinone detected in some commercial samples in our study, possibly due to refining or intensive processing steps." Abedinzadeh et al. (2024) reported that the amount of thymoquinone in black cumin oil obtained by cold pressing was 3013 mg/kg; However, this value dropped to only 130 mg/kg in the oil obtained by solvent extraction from the pulp after pressing.Interestingly, Neunert et al. (2025) observed that heating black cumin oil for three days in open and closed bottles resulted in a 25% increase in thymoquinone levels, which remained stable over time. This phenomenon is thought to be linked to the thermal conversion of monoterpenes such as thymol or carvacrol via a biosynthetic pathway (Elyasi et al., 2022; Ahmad et al., 2019). Furthermore, the accumulation of other volatiles and storage conditions may play a role in determining the final thymoquinone concentration. Considering the 180-fold difference observed in our samples, the data strongly suggest that some commercial oils may either be adulterated, poorly processed, or incorrectly labeled, which has direct implications for consumer trust and regulatory monitoring. These findings reveal that thymoquinone levels are strongly influenced not only by the origin of the seed but also by the extraction technique and processing steps applied, explaining the underlying reasons for the wide variation observed among commercial black cumin oils.

3.6. Change in Peroxide Value During Storage

The changes in peroxide values of black cumin oil samples over a 30-day storage period are shown in **Figure 2**. The oils exhibited varying oxidative behaviors during storage. A general increase in peroxide value was observed in samples A, B, E, H, and K throughout the storage period, suggesting a progression of lipid oxidation in these samples. The control sample (M) showed a decline in peroxide value until day 15; however, the value began to increase thereafter and reached 124.539 meq O_2/kg on day 30, making it the sample with the highest final peroxide

value. Interestingly, sample C exhibited a storage behavior similar to sample M, with a late-phase increase in oxidation products. In contrast, samples F and I maintained relatively stable peroxide levels until days 10–15, followed by a gradual decrease, ultimately showing the lowest peroxide values at the end of the storage period. Sample D showed a distinctive pattern: the peroxide value decreased to its lowest on day 10 and then steadily increased until day 30. Overall, except for samples F, I, and G, all other oils reached their maximum peroxide value by the end of storage. These fluctuations in peroxide values may be attributed to the dynamic nature of lipid oxidation, wherein primary oxidation products (hydroperoxides) are first formed, followed by their decomposition into secondary oxidation compounds. As Domínguez et al. (2019) noted, the accumulation and subsequent breakdown of peroxides can lead to non-linear oxidative trends. Supporting this, Oubannin et al. (2022) reported that peroxide values of black cumin oils stored at 60 °C for 120 days initially increased but decreased after one month, due to the degradation of hydroperoxides into secondary products. Similarly, Cuvelier and Maillard (2012) highlighted that a decline in peroxide value during extended storage may reflect the instability and conversion of primary oxidation products. In another study, Kiralan et al. (2014) found that cold-pressed black cumin oil stored at 60 °C for 6 days showed no change in peroxide value, but after 12 days of storage, the value increased by approximately 20%. Additionally, Ekici et al. (2018) reported that among various seed oils, black cumin oil had the highest initial peroxide value (26.05 meq O_2/kg), and after 12 months of storage at different temperatures, it also exhibited the greatest increase in both peroxide and specific absorbance values. These findings confirm that accelerated storage tests may reveal not only the initial oxidative status of oils but also their susceptibility to secondary degradation pathways, highlighting the complexity of stability evaluation.

3.7. Fatty Acid Composition of Black Cumin Oil Samples

The fatty acid composition of the black cumin oil samples is presented in **Table 2**. The palmitic acid (C16:0) contents of the samples ranged from 5.32% (sample K) to 10.91% (sample M). The palmitic acid levels of samples A, C, F, G, and I were similar to that of the control sample M, produced by cold pressing under laboratory conditions, and were higher than those of the remaining oils. Samples D and E followed in terms of palmitic acid content, while sample K exhibited the lowest level. Palmitic acid is said to be associated with an increased risk of dyslipidemia and cardiovascular disease, particularly due to its high saturated fatty acid content (Risérus, 2008; Vessby et.al., 2001).The stearic acid (C18:0) contents of the samples ranged from 3.24% to 4.27%. The highest level was found in sample B, which was statistically higher

than samples C, D, F, G, I, K, and M, but in the same statistical group as samples A, E, and H (**Table 2)**. The oleic acid (C18:1) content varied between 25.69% and 42.10%, with sample K showing the highest level (**Table 2)**. Oleic acid is a monounsaturated fatty acid that helps regulate glucose levels, lipid metabolism, and inflammatory responses, thereby reducing cardiovascular risk (Lopez et al., 2010). The highest linoleic acid (C18:2) content was detected in sample M (57.85%), followed closely by sample H. Eicosadienoic acid (C20:2) levels were highest in sample F (3.88%), while sample H contained 0.90%. Other samples showed similar eicosadienoic acid levels (**Table 2)**. Linolenic acid (C18:3) was detected only in samples B (11.62%) and C (0.53%). Arachidic (C20:0) and behenic (C22:0) acids were found exclusively in sample K (0.74% each), eicosenoic acid (C20:1) in sample D (0.60%), and docosenoic acid (C22:1) in sample C (1.55%). These fatty acids were not detected in other samples (**Table 2)**. Overall, the unsaturated fatty acids in the black cumin oil samples were mainly linoleic acid (48.30–57.85%) and oleic acid (25.69–42.10%), while the predominant saturated fatty acid was palmitic acid (5.32–10.91%) (**Table 2)**. These results are in accordance with previous studies. For example, Lutterodt et al. (2010) reported linoleic acid content between 58.8–61.2 g/100 g, oleic acid between 22.6–24.5 g/100 g, and palmitic acid between 13.0–13.3 g/100 g in cold-pressed black cumin oil. Hamrouni-Sellami et al. (2008) and Nickavar et al. (2003) found oleic acid levels ranging from 12.7% to 27.4% and palmitic acid levels from 9.9% to 18.4%, indicating that genetic and climatic factors significantly influence fatty acid composition (Cheikh-Rouhou et al., 2007). Abedinzadeh et al. (2024) and Agah et al. (2024) reported that the dominant fraction in cold-pressed Nigella sativa oils is linoleic acid, followed by oleic acid as the main component; palmitic acid stands out as the main saturated fatty acid. Similarly, Soltani et al. (2024) and Abedinzadeh et al. (2023) emphasized that although the extraction method and refining steps can partially alter the fatty acid ratios, the linoleic-oleic acid dominance is maintained. Supporting this, Telci et al. (2021) observed variations across harvest years, reporting linoleic acid contents of 54.10–57.50% in the first year and 48.60–57.80% in the second, while oleic acid ranged from 22.0–25.3% and 21.1–24.9%, respectively. Likewise, Aftab et al. (2014) and Kiralan et al. (2014) reported linoleic acid levels between 51.8–57.49%, oleic acid between 23.95–28.55%, and linolenic acid around 0.25%. The atypical detection of high linolenic acid in sample B and the presence of unusual long-chain fatty acids in sample K may indicate possible varietal differences or blending practices, which require further investigation.

4. Conclusions

In this study, significant variations were observed in the physicochemical characteristics of commercially available cold-pressed black cumin (Nigella sativa L.) oils. Notably, marked differences were found in peroxide values, free fatty acid levels, and thymoquinone concentrations among the samples. The detection of excessively high levels of oxidation products and free fatty acids in certain samples suggests insufficient quality control practices during production and storage. Furthermore, the approximately 190-fold variation in thymoquinone content may point to possible adulteration, degradation due to improper handling, or inefficient extraction techniques used in commercial production. Analysis of the fatty acid composition revealed that the oils were generally rich in linoleic and oleic acids, consistent with the known profile of black cumin oil. However, substantial differences were noted in terms of oxidative stability and shelf-life potential, reflecting disparities in production conditions, seed quality, and storage practices. Currently, the Turkish Food Codex does not include specific quality standards for cold-pressed black cumin oils. The lack of regulatory criteria and enforcement mechanisms contributes to inconsistencies in product quality and poses potential risks to consumer health and product authenticity. Therefore, establishing legally binding regulations and standardized analytical benchmarks, particularly for peroxide value, free fatty acidity, and thymoquinone concentration, is crucial. In addition to physicochemical analyses, sensory evaluation of cold-pressed oils should also be considered as an important complementary quality indicator for the standardization of black cumin oil. Incorporating sensory assessment alongside analytical parameters may contribute to a more comprehensive evaluation of product quality, authenticity, and consumer acceptance. Future studies should also focus on the development of rapid authentication techniques and long-term monitoring of product stability under real storage conditions, to further support consumer safety and industry transparency. The findings quantitatively demonstrate that products on the market exhibit unpredictable differences in terms of shelf life and functional value, and clearly reveal that establishing standardized processes, traceability, and binding quality criteria throughout the production-to-consumption period for black cumin oil is a scientific necessity.

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Table 1. Peroxide value, free fatty acidity, induction period and thymoquinone values of different black cumin oil samples.[a]

Samples	n	Peroxide value (meq O /kg)	Free fatty acid (% Oleic acid)	Induction time (h)	Thymoquninoe (mg/g oil)
A	3	38.87±2.28^{D}	9,64±0.13^{C}	8.57± 0.13^{D}	3.79±0.03^{F}
B	3	13.70±1.38^{G}	4,71±0.16^{E}	3.99± 0.16^{F}	0.63±0.01^{H}
C	3	88.57±2.16^{A}	3,01±0.56^{F}	9.49± 0.56^{D}	19.76±0.42^{A}
D	3	69.96±2.04^{B}	6,12±0.38^{D}	4.80± 0.38$^{E\ F}$	12.58±0.43^{C}
E	3	20.35±2.12^{F}	12,9±0.03^{B}	2.85±0.03^{G}	1.33±0.06^{G}
F	3	51.32±1.07^{C}	0.00±0.28^{G}	15.82±0.28^{A}	0.11±0.01^{H}
G	3	42.71±1.58^{D}	4,13±0.13^{E}	11.00±0.13^{C}	3.71±0.08 ^{F}
H	3	9.84±0.7 ^{G}	0,66±0.55^{G}	5.14±0.55^{E}	0.13±0.06^{H}
I	3	53.45±0.90^{C}	2,57±0.60^{F}	14.33±0.60^{B}	9.99±0.08^{D}
K	3	24.57±2.42^{F}	18,53±0.31^{A}	1.76±0.30^{H}	19.15±0.02^{B}
M	3	29.85±1.36^{E}	4.57±0.37^{E}	9.22±0.37^{D}	4.75±0.07^{E}

[a] Means followed by the different letters within a column are significantly (P< 0.05) different.

Table 2. Fatty acid composition results (%) of different black cumin oil samples.

Fatty acid	A	B	C	D	E	F	G	H	I	K	M
Palmitic acid (C 16:0)	10,37±0,01^{B}	7,2±0,06 ^{D}	10,6 ±0,02^{A}	10,02±0,11^{C}	9,94 ±0,07^{C}	10,5±0,01^{AB}	10,54±0,01^{AB}	7,68±0,04 ^{D}	10,63±0,20 ^{A}	5,32±0,04^{E}	10,91±0,03^{A}
Stearic acid (C 18:0)	3,81 ±0,03^{AB}	4,27±0,02 ^{A}	3,24±0,13 ^{D}	3,68±0,01^{BC}	3,86±0,01 ^{AB}	3,55 ±0,12^{C}	3,66±0,02 ^{BC}	3,8±0,1 ^{AB}	3,49±0,04 ^{CD}	3,55 ±0,02^{C}	0,03±0,1^{E}
Oleic acid (C 18:1)	27,99±0,01^{CD}	25,69 ±0,04^{F}	27,59±0,03^{CD}	29,55 ±0,03^{B}	28,43±0,08 ^{C}	26,57±0,03^{EF}	27,35±0,01^{DE}	30,39±0 ^{B}	27,5±0,03^{CDE}	42,1±0,13^{A}	28,04±0,11^{CD}
Linoleic acid (C 18:2)	54,96±0,02^{BC}	50,09±0,01 ^{E}	52,89 ±0,04^{D}	53,22±0,01^{D}	54,52±0,1 ^{C}	55,48±0,01^{B}	54,84±0,13^{BC}	57,24±0,01 ^{A}	54,96±0,22^{BC}	48,3 ^{F}	57,85 ±0,04 ^{A}
Linolenic acid (C 18:3)	N.D	11,62±0,04 ^{A}	0,53 ±0,05^{B}	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Arachidic acid (C 20:0)	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.74	N.D
Eicosenoic acid (C 20:1)	N.D	N.D	N.D	0.60±0,02	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Eicosadienoic acid (C 20:2)	2,88 ±0,02 ^{C}	1,13±0,03 ^{D}	3,6±0,05 ^{A}	2,93 ±0,04^{BC}	3,26 ±0,01^{BC}	3,88 ±0,02^{A}	3,62±0,21 ^{AB}	0,9±0 ^{D}	3,41 ±0 ^{AB}	N.D	3,17 ±0,01^{BC}
Behenic acid (C 22:0)	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.74	N.D
Docosenoic acid (C 22:1)	N.D	N.D	1,55 ^{A}	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D

N.D: Not Detected.

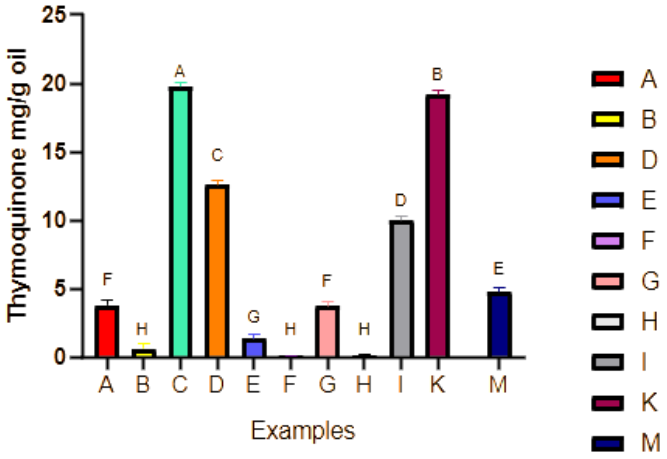

Figure 1. Thymoquinone amounts of different black cumin oil samples.

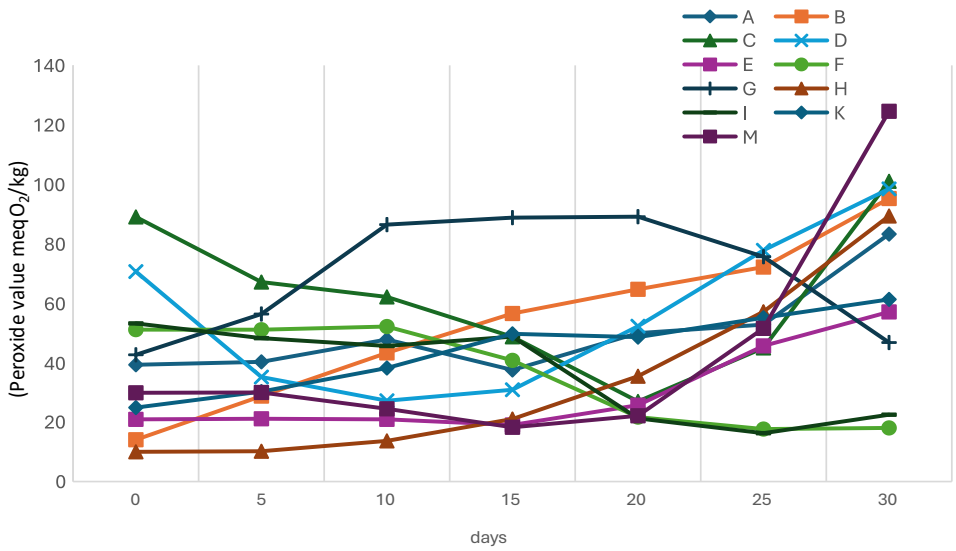

Figure 2. Effect of storage time on peroxide values of analyzed black cumin oils.