A Case Study on Profile Investigation of Cold-Pressed Black Cumin Seed Oil Produced in Turkey

Türkiye'de Üretilen Soğuk Sıkım Çörek Otu Tohum Yağı Profilinin İncelenmesine İlişkin Bir Durum Çalışması

Research Article

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ABSTRACT

Nigella sativa L. (black cumin seed) is an extensively used medicinal plant all around the world. Its chemical composition contains many pharmacologically active components. In this study, *Nigella sativa* L. seeds originated from Mersin, Turkey and oil was obtained using cold-press technique. Chemical composition of cold-pressed black cumin seed oil (BCSO) was utilized in terms of sterol composition, fatty acids composition, triacylglycerols, thymoquinone and physical properties of refractive index and color were evaluated. The major sterols were β-Sitosterol, followed by sitostanol, Δ5-avenasterol and Δ7-avenasterol. The major unsaturated fatty acids were linoleic acid and oleic acid while the major saturated fatty acid was palmitic acid. The most founded triacylglycerol components are LLL, OOLn+PoOL, POL+PoPoPo+PPoL, PoOO and PoOP+SPoL+SOLn+SPoPo. Concentration of the thmoquinone in the cold-pressed black cumin seed oil was calculated as 10099±117 mg/kg. Refractive index was measured at three different temperatures (20°C, 25°C and 40°C) and Red (R), Blue (B), Yellow (Y) and Neutral (N) colours were measured with tintometer. The results from the proposed study that cold-pressed black cumin seed oil may supply as a brilliant nutritional source of thymoquinone and natural antioxidants. In terms of oil major components, composition of Nigella seed oil from Mersin, Turkey is similar with other countries and other cities from Turkey which are given literature although some chemical and physical components were shown differences according to agronomical conditions and extraction techniques.

Key Words
Black Cumin seed oil, Sterol, Fatty Acids, Triacylglycerides, Thymoquinone, Colour, Refractive Index.

ÖZ

Farmakolojik olarak aktif bir kimyasal kompozisyona sahip olan *Nigella sativa* L. (kara çörek otu) dünya çapında geniş kullanım alanı olan tıbbi bir bitkidir. Bu çalışmada, Mersin orjinli *Nigella sativa* L. tohumlarının yağları soğuk sıkım tekniği ile elde edilmiştir. Soğuk sıkım kara çörek otu yağının (KÇOY) kimyasal içeriği, sterol kompozisyonu, yağ asitleri kompozisyonu ve triaçilgliseroller, fiziksel özellikleri ise kırılma indisi ve renk özellikleri açısından değerlendirilmiştir. Başta β-sitosterol olmak üzere, sitostanol, Δ5-avenasterol ve Δ7-avenasterol en fazla bulunan sterollerdir. Doymamış yağ asitleri olarak linoleik ve oleik asit, doymuş yağ asitleri olarak da palmitik asit en fazla bulunan yağ asitleridir. En çok bulunan triaçilgliserol bileşenleri sırasıyla, LLL, OOLn+PoOL, POL+PoPoPo+PPoL, PoOO ve PoOP+SPoL+SOLn+SPoPo bileşenlerdir. Timokinon miktarı 10099±117 mg/kg olarak hesaplanmıştır. Kırılma indisi değerleri üç farklı sıcaklıkta (20°C, 25°C and 40°C) ve renk değerleri ise kırmızı (K), mavi (M), sarı (S) ve notr (N) olmak üzere dört farklı renk skalasında değerlendirilmiştir. Bu çalışmalar sonucunda kara çörek otu yağının timokinon ve antioksidan içeriği bakımından ne kadar muhteşem bir besin kaynağı olduğu anlaşılmaktadır. Yağda en çok bulunan bileşenler bakımından Mersin orjinli bu yağın, diğer ülkelerdeki ve Türkiye'nin diğer şehirlerindeki yağlara benzer kimyasal ve fiziksel özellikler içerdiği, farklanmaların ise yağ özütleme tekniklerine ve agronomik koşullara bağlı olduğu anlaşılmıştır.

Anahtar Kelimeler
Çörek Otu tohumu yağı, Sterol, Yağ Asitleri, Triaçilgliseroller, Timokinon, Renk, Kırılma İndisi.

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INTRODUCTION

Nigella sativa L. is an annual herbal plant belonging to the Ranunculaceae family growing in countries bordering the Mediterranean Sea [1]. The seed of *Nigella sativa,* known as black seed and black cumin has been used for nutritional, industrial and medicinal purposes in recent years [2]. Black cumin seed (BCSO) has an extensive beneficial effects on health such as asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness, and influenza [3,4]. Some of reported pharmacological properties of BCSO are: antioxidant effect, anti-inflammatory and analgesic actions, anti-carcinogenic and mutagenic activity, anti-hepato and nephrotoxic action, respiratory and immunological actions, anti-diabetic action, effect on cardiovascular system and blood, anti-ulcer action, antimicrobial actions, antiparasitic actions [5-9]. Most of these activities have been related quinone constituents [10]. Thymoquinone is the most important biologically active molecule which shows pharmacological effects on hepatotoxicity, cardiotoxicity, diabetes mellitus, bronchial asthma, and gastric mucosal injury [11]. In addition, the main constituents of black cumin seeds are alkaloids, volatile and fixed oils. nigella seeds contain high amounts of linoleic, oleic and palmitic acids as a fixed oil. Besides of these fatty acids, it contains highly amount of tocopherols which is naturally antioxidant compound and bioactive sterols [12,13]. Volatile oil contains 32 compounds notably trans-anethole, p-cymene, limonene, carvone and thymoquinone [12]. There are several studies about isolation and identification of active constituents in volatile oil nigella seeds.

Over the last few years cold pressing (mechanical pressing) technology has been used for seed oil production, which involves no heat treatment or solvents [14]. This technique is less expensive and less labor-intensive than the extraction methods using chemicals. Increased interest in cold pressing technology due to this application is safe, simple, advantageous, efficent, native process and ecologically friendly [3,15]. Cold-pressed oils have higher levels of natural antioxidants and thymoquinone derivates than other extracted oils.

Some physicochemical properties were studied to indicated quality of black cumin seed oil in many studies. Refractive index, colour, free fatty acids as oleic acid %, peroxide value, iodine value, absorption in UV (232 nm and 270 nm), saponification number, specific gravity were supplied quality and purity control of oils [16-18].

Therefore, the main aim of the present study was determining the fatty acid profile, sterol composition, triacylglycerol composition, thymoquinone content, absorption in UV at two different wavelengths (232 and 270 nm) and physicochemical characteristics such as refractive index and colour of cold-pressed black cumin seed oil as a case study. The results of this study will be used to determine properties of BCSO and results of analyses can be used to compare with literatures from another location both Turkey and other countries.

MATERIALS and METHODS

Oil Sample

BCSO obtained by cold-press extraction (CPE) were kindly donated from EGE-LS (Turkey). Black cumin seeds were ensured Mersin, which are a city of Mediterranean region at the Turkey.

Reagents

The reagents used were either analytical or chromatographic grade. Millipore ultrapure water (Type I) was used for all analysis. All reagents were obtained by Sigma-Aldrich, Merck, Supelco and LabScan and the grade of the all other chemicals and solvents were either chromatographic or analytical.

Sterol Analysis

The analysis of the sterols in BCSO sample was performed according to the official gas chromatographic method described in the COI/T.20/Doc. No.10/ Rev.1 [19]. For the determination of sterol compositions, the method describes a procedure for determining the individual and total sterols content of fatty substances within BCSO.

Chromatographic analysis of sterol compositions was performed by a Thermo Finnigan

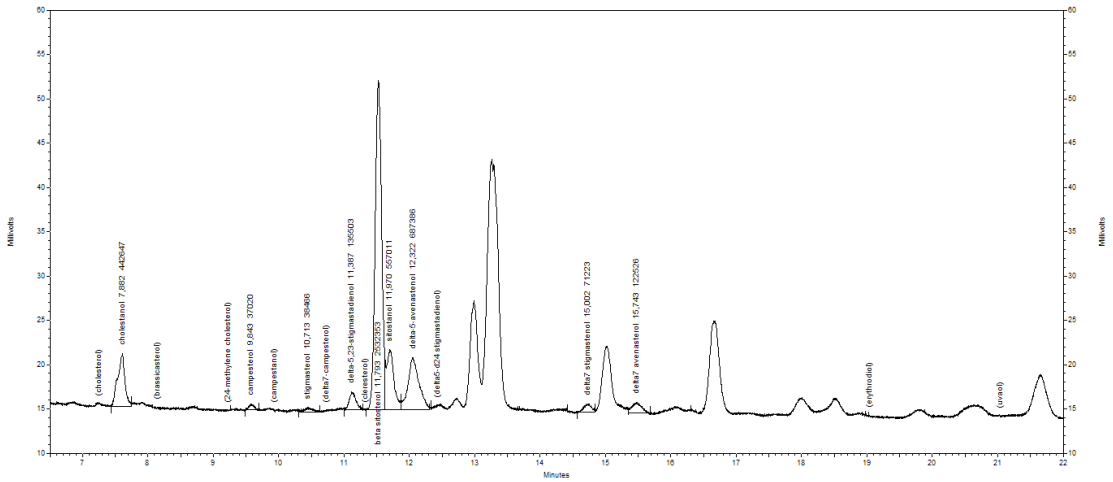

Figure 1. GC-FID chromatogram of sterol compositions in the BCSO.

GC-FID system with Zebron ZB-5ms capillary column (30 m x 0.25 mm ID x 0.25 μm). Split ratio was performed as 1:50 for the instrument. Injection port and detector temperatures were 250°C and 290°C, respectively. The oven temperature program was run at 260°C as isothermal during the analysis. Retention times of each sterol peak were identified as regard relative retention time of β-Sitosterol which specified at testing methods of International Olive Council (IOC) [19]. Sample chromatograms of sterol compositions of BCSO was given in the Figure 1.

Peak quantification is expressed as according to α-cholestanol (5α-Cholestan-3β-ol,≥95%) using by internal standard according to sterol testing methods of IOC.

Fatty Acid Methyl Esters (FAME) Analysis

The composition of fatty acid methyl esters (FAMEs) was determined according the EU Regulation 2568/91 (European Union Commission, 1991); COI/T.20/Doc. 24-2001 "preparation of the FAMEs from olive oil and olive pomace oil", COI/T.20/Doc. No 33 and COI/T.20/Doc.17/Rev. 1-2001 "determination of trans unsaturated fatty acids by capillary column gas chromatography" [19]. The chromatographic separation was performed by an Agilent GC-FID system, equipped with HP-88 column (60 m×0.25 mm, 0.20 μm). The oven initial temperature was held at 140°C for 1.0 min and then increased 240°C at a rate of 4°C min^{-1}, where the temperature was held for 5 min. Methyl esters are formed by trans-esterification with cold methanolic solution of potassium hydroxide. FAME chromatogram was given in the Figure 2.

Identification of the fatty acids is made according to their retention times. Thirty-seven component mixture of FAME was used as the standard for determination of retention time of fatty acids. The results were expressed as peak area (relative) percent.

Triacylglycerol (TAG) Analysis

The analysis of the TAG profile in BCSO sample was performed according to the official HPLC method described in the COI/T.20/Doc. No.20/ Rev.2 and COI/T.20/Doc. No. 25/Rev. 1 [19]. The analysis was carried out in an Agilent 1100 HPLC system with refractive index detector (RID). The quantitative results were obtained by expressing the area of a given peak as a percentage of the sum of the areas of all the peaks. The column was a Phenomenex Luna 5μm C18 100A 250*4.60 mm HPLC column. The mobile phase was propionitrile delivered isocratically at a flow rate of 1.5 mL min^{-1} at 30°C column temperature. TAG composition chromatogram was shown in Figure 3.

Abbreviations of nomencleature of TAG, where L, O, P, Ln, S, and Po are linoleic, oleic, palmitic, linoleic, stearic, palmitoleic acids, respectively.

Thymoquinone (TQ) Analysis

Quantification of the TQ in BCSO was achieved using an Agilent 1100 RP-HPLC system consisting

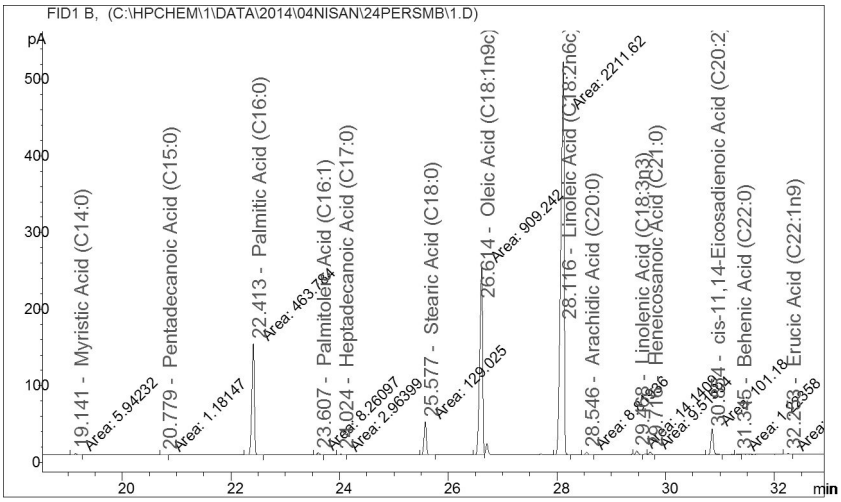

Figure 2. GC-FID chromatogram of FAME compositions in the BCSO.

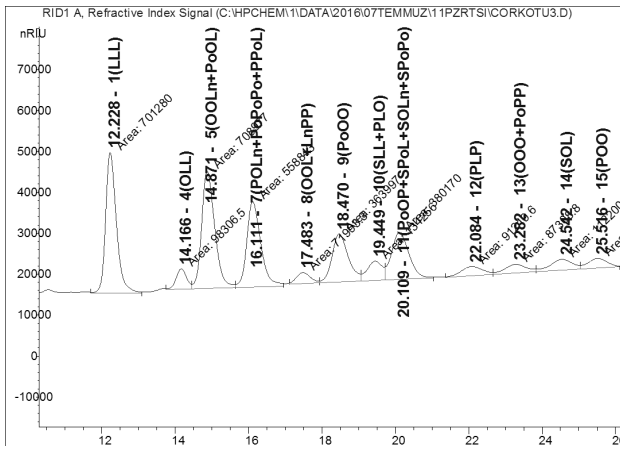

Figure 3. HPLC-RID chromatogram of TAG compositions in the BCSO.

of a gradient pump, a DAD detector, and a C18 column (Agilent Eclipse column XDB-C18 (5.0 μm particle size, 4.6x150 mm)). Quantitative analysis was based on the peak areas. Detection and quantification were carried out at 254 nm. The column temperature was 25°C. The mobile phase consisted of methanol: water: methyl tert-butyl ether (50:40:10; v/v/v) with a flow rate of 1.0 mL min^{-1}. To detect TQ in the BCSO, 0.1 g of BCSO was dissolved in 10 mL of hexane, vortexed for 1 min, and filtered; then 20 μL was injected into the HPLC system. HPLC-DAD sample chromatogram of TQ was shown in Figure 4.

Linearity, Limit of Detection (LOD), Limit of Quantification (LOQ) parameters of analytical method validation were given Table 1.

There is a good correlation between concentration and peak area according to correlation efficient for purposed methods in this study. LOD and LOQ values were shown this method has a good sensitivity. Three concentrations were selected for accuracy parameters for method validation. Table 2 was shown mean recovery values of methods.

According to percent of accuracy this proposed methods is reliable, accurate and could be used for thymoquinone analysis.

Determination of Refractive Index and Colour

Refractive index value was measured by using Atago RX-7000 instrument. Refractive index was measured 25°C and 40°C. Lovibond PFX 880

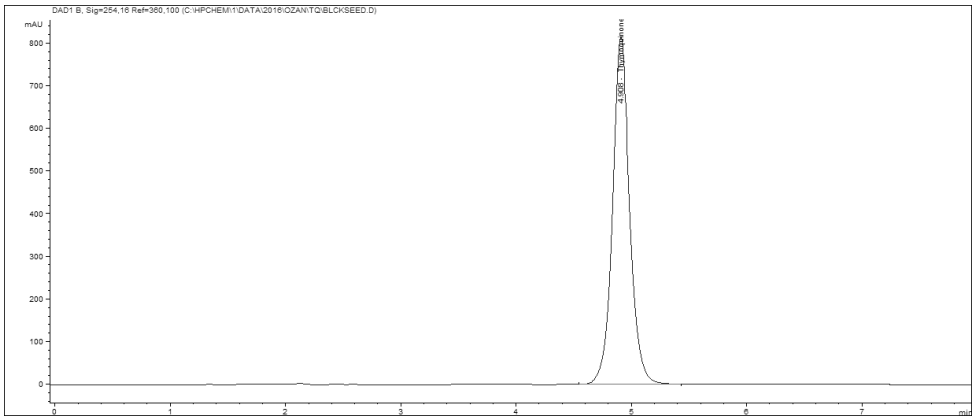

Figure 4. HPLC-DAD chromatogram of TQ in the BCSO.

Table 1. Linearity and Limit of detection (LOD) and Limit of Quantification (LOQ) values of TQ.

HPLC method	Thymoquinone
Linear Ranges (μg/g)	1.0 – 25
Linear Regression Equation	y= 135.39x+43.943
Correlation Coefficient	0.9998
Retention Time (min)	4.908
LOD	0.01
LOQ	0.04

Table 2. Accuracy value of TQ for three different concentrations.

Concentration (μg/g)	Mean Recovery (%) ±SD	RSD (%)
2.50	94.64±1.96	2.07
5.00	93.51±2.08	2.23
10.00	92.36±3.39	3.67

tintometer was used for colour analysis. Red (R), Blue (B), Yellow (Y) and Notr (N) colours were measured with tintometer.

RESULTS and DISCUSSION

Sterol Composition of BCSO

Sterol compositions in seed oils are used to identification of oil and to determination of oil quality [20]. In addition, level of sterol composition gives knowledge about authentication and adulteration of oil [21]. Because of, sterol composition values of cold-press BCSO can be given knowledge used to have knowledge for Mersin location. Eight sterols and total sterol were evaluated in this study as they can be seen in Table 3.

β-Sitosterol was major sterol in the oil sample (62.24%) followed by Δ5-Avenastenol and Sitostanol ranged 16.33% and 11.65%, respectively. Campesterol, Stigmasterol, Δ5-23 Stigmastadienol, Δ7-Stigmastenol, Δ7-Avenasterol were also founded in the oil sample. BCSO can be used a source of β-Sitosterol which has to inhibit the absorption of dietary cholesterol [22]. When the authors compared the results of Atta, 2003 and Kiralan et al., 2014 previous studies, which include the results of sterol from Turkey (Konya) and Egypt, the authors concluded that BCSO has got similar sterol compositions, in addition to this, some sterol parameters were shown differences according to amount and type [13,16]. Cholesterol was only detected cold-press

Table 3. Sterol % compositions of cold-press black cumin seed oil (BCSO) (%) in this study and references.

STEROL	This Study Cold Press Mersin/ Turkey (%)	[16] Local Market Konya/ Turkey (%)	[23] Local Market İzmir/ Turkey (%)	[24] Tunisian (g/100 g)	[24] Iranian (g/100 g)	[13] Egypt CPO (mg/100 g)	[25] Moroccan CPO (%)
Campesterol	0.89±0.10	14.88±0.19	11.9±0.99	13.76±0.25	12.09±0.11	28±6	13.1±0.5
Stigmasterol	1.10±0.19	17.48±0.54	18.6±1.52	20.92±0.24	16.57±0.22	68±11	17.8±0.5
Δ5-23 Stigmastadienol	3.09±0.24	-	-	-	-	-	-
β-sitosterol	62.24±1.03	58.05±1.01	69.4±2.78	44.53±0.24		636±35	49.4±1.5
Sitostanol	11.65±0.94	-	-	-	-	44±10	-
Δ5-Avenasterol	16.33±0.33	7.27±0.74	-	-	-	-	12.4±0.5
Δ7-Stigmasterol	1.78±0.15	1.24±0.10	-	2.22±0.11	1.60±0.20	-	0.6±0.1
Δ7-Avenasterol	3.19±0.18	1.62±0.31	-	2.24±0.19	1.17±0.19	-	2.1±0.2
Cholesterol	ND*	-	-	1.28±0.01	0.93±0.04	52±9	0.9±0.1
Total sterol (g/kg)	1.92±0.04	NC**	NC	2.81±0.12	2.58±0.18	NC	-

*ND: not detected **NC: not calculated. The results were shown as mean±SD. SD: standart deviation.

BCSO from Egypt. There are differences at the proportion of campesterol, stigmasterol between Mersin and Konya from Turkey. Δ5-Avenastenol, Δ7-Stigmasterol, Δ7-Avenasterol were only found in this study and Kiralan et al, 2014 from Turkey compared with previous study of Atta, 2003 from Egypt. Δ5-23 Stigmastadienol and sitostenol were detected in the BCSO where are used in this study from Mersin. Nergiz and Otles, 1993 were just found campesterol, stigmasterol and β-Sitosterol in the BCSO from İzmir (Turkey) [23]. Cheikh-rouhou et al., 2008 analyzed sterol composition of BCSO from Tunisian and Iranian [24]. Total sterols were founded as 2.81±0.12 (g/kg) in the 2.58±0.18 (g/kg) in Tunisian and Iranian oils, respectively. Amount of total sterol which was calculated BCSO from Mersin was as 1.92±0.04 (g/kg). Level of β-Sitosterol was higher than Tunisian and Iranian oils. BCSO of Tunisian and Iranian were included cholesterol likewise oil from Egypt. Δ5-23 Stigmastadienol was only contained as percent of 3.09±0.24 in the oil from Mersin. Gharby et al., 2015 were studied cold press BCSO from Morocco and their results were shown similarity with other studies [25]. Amount of Campesterol and stigmasterol has lowest percent in the oil which analysed this study. When the results of sterol content were utilized it was shown there are differences between both cities from same country and other countries therefore data was used to authentication of cold-pressing BCSO.

Fatty Acid Methyl Esters (FAME) Analysis

FAME analysis was given information about purity and quality of oils. Sixteen fatty acid methyl esters was detected in this study and values of FAME were given in Table 4 which was included results of other study from another city of Turkey and another country.

When the data was evaluated on the Table 4 of FAME composition of BCSOs were shown similarities but there are some differences between amount of FAME. While level of Myristic Acid (C14:0), Linolenic acid (C18:3) and Arachidic acid (C20:0) were highest in the oil from Egypt, Oleic acid (C18:1), and Linoleic acid (C18:2) were been lowest at the Atta MB (2003) study [13]. When this study was compared with BCSO of Tunisian and Iranian were high in terms of Palmitic Acid (C16:0) and Behenic acid (C22:0)

Table 4. FAME % compositions of cold-press black cumin seed oil (BCSO) in this study and references.

FAME (%)	This Study Cold Press Mersin Turkey	Local Market Konya Turkey [16]	Local Market İzmir Turkey [23]	Moroccan [25]	Tunisian (g/100 g) [26]	Iranian (g/100 g) [26]	Egypt CPO [13]
C14:0	0.15±0.01	0.13±0.00	1.2±0.04	1.0±0.01	0.35 ± 0.02	0.41 ± 0.05	11.1±1.1
C16:0	11.94±0.13	12.01±0.09	11.4±1.00	13.2±0.2	17.2 ± 0.15	18.4 ± 0.25	12.11± 3.4
C17:0	0.07±0.01	0.06±0.00	-	-	-	-	-
C18:0	3.32±0.02	2.77±0.09	2.90±0.24	2.3±0.1	2.84 ± 0.08	3.69 ± 0.12	3.7±1.7
C20:0	0.23±0.01	0.15±0.00	-	0.4±0.1	0.14 ± 0.02	0.22 ± 0.01	1.2±0.8
C21:0	0.24±0.00	-	-	-	-	-	-
C22:0	0.03±0.01	-	-	-	1.98 ± 0.08	2.60 ± 0.05	-
C24:0	-	0.31±0.02	-	-	-	-	0.2±0.1
C16:1	0.21±0.01	0.25±0.01	-	0.2±0.1	1.15 ± 0.05	0.78 ± 0.25	0.5±0.1
C17:1	-	0.03±0.00	-	-	-	-	-
C18:1	23.78±0.47	23.95±0.12	21.9±1.00	23.8±0.1	25.0 ± 0.24	23.7 ± 0.06	18.9±5.4
C20:1	-	0.27±0.01	-	-	0.32 ± 0.04	0.34 ± 0.05	-
C22:1	0.07±0.01	-	-	-	-	-	0.7±0.4
C18:2	56.94±0.34	57.49±0.08	60.80±2.67	58.5±0.1	50.31 ± 0.25	49.15 ± 0.06	47.5±6.5
C18:3	0.36±0.01	0.25±0.00	-	0.4±0.1	0.34 ± 0.06	0.32 ± 0.05	2.1±0.4
C20:2	2.59±0.02	2.33±0.04	1.70±0.11	-	-	-	-

The results were shown as mean±SD. SD: standart deviation.

according to Cheikh-rouhou et al., 2007 studies [26]. Heptadecanoic acid (C17:0) was detected in this study and BCSO from Konya. Heneicosanoic (C21:0) was shown only in the oil at this study. Fatty acids composition did not show a noticeable differentiation.

Triacylglycerol (TAG) Analysis

Triacylglycerols were the major part of saponifiable matter in the oils. Twelve TAGs were detected in the BCSO as it can be shown in the Table 5. The best knowledge of ours and literature survey of the authors there are only two studies interested with TAG composition of BCSO. Khoddami et al., 2010 and Ramadan and Morsel, 2002 were studied solvent extraction of BCSO [27]. Ramadan and Morsel quantitatively analyzed of TAG composition by GC-FID system therefore results of this study was compared with hexane extract results from Khoddami et al, 2010 [28].

LLL 20.60 ± 0.47, OLL 16.58 ± 0.40, PLL 18.51 ± 0.58, OOL 9.48 ± 0.23, POL 13.99 ± 0.34, POO 1.35 ± 0.06 and PPO 1.75 ± 0.39 were found in the prior study of Khoddami et al., 2010 [27]. LLL, OLL, POO were common TAGs for two studies. LLL was shown about same in the studies. Value of OLL was very higher than this study. POO is lower than this work. When the authors compared with POL+PoPoPo+PPoL values from cold-press and POL values from solvent extraction the authors were shown that there is only small differentiation between them. This could have occurred because of PoPoPo+PPoL. According the results of these three components there are some differences between composition of TAG but this variation could be happened from extraction techniques.

Table 5. TAG % compositions of cold-press black cumin seed oil (BCSO) in this study.

TAG*	1	2	3	4	5	6	7	8	9	10	11	12
CPO	20,75 ±0.16	2,95 ±0.11	20,76 ±0.23	15.96 ±0.49	2,21 ±0.19	10,79 ±0.36	4,00 ±0.05	11,12 ±0.18	2,83 ±0.15	2,62 ±0.04	3,37 ±0.18	2,67 ±0.13

The results were shown as mean±SD. SD: standart deviation.
*TAG No: 1.LLL, 2. OLL, 3. OOLn+PoOL, 4. POL+PoPoPo+PPoL 5. OOL+LnPP, 6. PoOO, 7. SLL+PLO, 8. PoOP+SPoL+SOLn+SPoPo, 9. PLP, 10. OOO+PoPP, 11. SOL, 12. POO.

Thymoquinone (TQ) Analysis

Concentration of TQ was calculated 10099±117 mg/kg (10.10±0.12 mg/g) in the BCSO from Mersin, Turkey. Al-Saleh I et al., 2006 studied 115 samples 25 from Al-Qassim Saudi Arabia 30 from Ethiopia, 25 from India, 5 from Sudan and 30 from Syria and average amount of thymoquinone were found 3098.5±1519.66 mg/kg (Ethiopia), 2362.68±1320.29 mg/kg (India), 2250.56±1923.55 mg/kg (Saudi Arabia), 1371.9±1381.5 mg/kg (Syria), 1274.6±60.67 mg/kg (Sudan) in their study [12]. Lutterodt et al., 2010 analyzed six different batch of cold pressed BCSO. Their results were expressed as mg/g oil. The lowest and highest concentrations of batches were calculated 3.48±0.19 and 8.73±0.53, respectively [14]. Solati et al., 2014 compared to find quantity of thymoquinone in BCSO from Iran which were obtained from soxhlet and super critical carbondioxide (SC-CO_2) extraction techniques and according to their results, amount of TQ were found 1.06±0.00 (mg/g) and 4.07±0.00 (mg/g) in oil from soxhlet and SC-CO_2 extraction, respectively [29].

When the compare quantity of TQ with previous studies, concentration of TQ in BCSO was found highest in this study. The reason of this could be extraction techniques, geographical origin and analytical methods. Amount of TQ could be distinguished BCSO from different country or locations and can give information about extraction techniques.

Determination of Refractive Index and Color

Refractive index (RI) value of BCSO was founded 1.4738±0.0000,1.4719±0.0000and1.4663±0.0000 at 20˚C, 25˚C and 40˚C, respectively. The range of refractive index were reported as 1.467- 1.491 in previous studies [13,26,27]. Atta, 2013 was reported results of RI in order of 1.4732±0.0001 and 1.4721±0.0002 for cold-pressed and solvent-extraction of BCSO [13]. When the compared with results of this study cold pressed oils were shown similar results at 20˚C but solvent extraction oil was act as at 25˚C. RI value of BCSO (solvent extraction) Tunisian and Iranian were measured as 1.47±0.01 and 1.46±0.01 at 40˚C by Cheikh-Rouhou et al., 2007 [26]. The result of this study lower than Tunisian and Iranian oils. In the earlier study of the Khoddami et al, 2010, BCSO were extracted using three different solvents for three methods which were the soxhlet, hexane, and Modified Bligh–Dyer methods and their results were 1.467±0.00, 1.468±0.00 and 1.491±0.00 at 25˚C for methods [27]. When the authors utilized results at different temperature and extraction procedure, refractive index value was shown more differences according to extraction techniques.

Results of color test was measured as Red (R) 30.0, Yellow (Y) 70.0, Blue (B) 0.0, Notr (N) 0.5. Color analysis of BCSO which was extracted with cold-pressed (R 08 Y 42 B 14) and solvent extraction (R 11 Y 42 B 81) was founded by Atta [13]. Color of BCSO from Tunisian and Iranian were shown at the CieLab coordinate by Cheikh-Rouhou, 2007 et al. and Yellow pigment was shown similarities with this study [26].

CONCLUSION

The present investigation focused on cold-pressed BCSO from Mersin, Turkey. BCSO has bioactive compounds which show positive effects in terms of on the human health. BCSO has high thymoquinone concentration therefore it could be used as natural supplement product in human diet. BCSO could be utilized a lot of area such as industrial, cosmetic and pharmaceutical. In addition, this study was revealed to comparison of BCSO with other city of Turkey and other countries in this way similar and different properties were

come out. Therefore, this data could be used for authentication and adulteration of BCSO otherwise effect of extraction techniques on the BCSO were shown with other studies.

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CONFLICT OF INTEREST

The authors have no competing interests to disclose.

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