

Physiochemical properties of Saudi *Nigella sativa* L. ('Black cumin') seed oil[☆]

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Abstract – The seeds of *Nigella sativa* L. (Ranunculaceae), commonly known as black cumin seeds and *Habat Al-barkah* in Saudi Arabia, are used extensively for flavouring and medicinal purposes. This work reported the study of physiochemical properties of Saudi black cumin seed oil (BCSO). The results of hexane extraction showed that black cumin seeds are rich in oil (43.7%). All the oil samples show high saponification values and low unsaponification values. High iodine values (122.7 mg/100 g) showed high degree of unsaturation (86%) for Saudi BCSO with about 60% of dominant linoleic acid (C_{18:2}). The volatile compounds presence in Saudi BCSO were extracted using steam distillation method and analyzed using gas chromatography mass spectrometer (GC/MS). The results showed that the volatile compounds such as *p*-cymene (31.50 ± 0.51%) and thymoquinone (25.35 ± 0.37%) were dominantly high in Saudi BCSO. Other compounds such as 3,5-dimethyl cyclohexanol, α -thujene, carvacrol, paeonol and longifolene were also present. Vitamin E was analyzed using High performance liquid chromatography (HPLC)-fluorescence method. The total concentrations of vitamin E were 451 ppm. The Saudi BCSO showed high content of linoleic acid (59.8%). The HPLC results showed that major triacylglycerols (TAGs) of Saudi BCSO were 1-oleoyl-2,3-dilinoleoylglycerol (OLL; 37.7 ± 0.4%) and 1,2,3-trilinoleylglycerol (LLL; 35.9 ± 0.3%). On the other hand, minor TAGs present were 1-palmitoyl-2,3-dilinoleylglycerol (PLL; 6.7 ± 0.9%), 1,2,3-trioleoylglycerol (OOO; 6.4 ± 0.5%) and 1,2-dioleoyl-3-linoleylglycerol (OOL; 5.7 ± 1.1%). The Saudi BCSO exhibited specific physicochemical properties and might be used for medicinal applications.

Keywords: Saudi BCSO / *p*-cymene and thymoquinone / vitamin E / linoleic acid

Résumé – Propriétés physicochimiques de l'huile de graines de *Nigella sativa* L. saoudienne. Les graines de *Nigella sativa* L. (Ranunculaceae), communément appelées graines de cumin noir et *Habat Al-barkah* en Arabie Saoudite, sont largement utilisées à des fins aromatiques et médicinales. Ce travail étudié les propriétés physico-chimiques de l'huile de graines de nigelle saoudienne (BCSO). Les résultats de l'extraction à l'hexane ont montré que les graines de cumin noir sont riches en huile (43,7%). Tous les échantillons d'huile présentent des valeurs de saponification élevées et des valeurs de non-saponification faibles. Des indices d'iode élevés (122,7 mg/100 g) ont montré un degré d'insaturation élevé (86 %) pour la BCSO saoudienne avec environ 60 % d'acide linoléique (C_{18:2}). Les composés volatils présents dans la BCSO saoudienne ont été extraits par distillation à la vapeur et analysés par chromatographie en phase gazeuse avec spectromètre de masse (GC/MS). Les résultats ont montré que les composés volatils tels que le *p*-cymène (31,50 ± 0,51 %) et la thymoquinone (25,35 ± 0,37 %) étaient majoritairement présents dans la BCSO saoudienne. D'autres composés tels que le 3,5-diméthyl cyclohexanol, le α -thujène, le carvacrol, le paeonol et le longifolène étaient également présents. La vitamine E a été analysée par chromatographie liquide à haute performance (CLHP) – méthode de fluorescence. Les concentrations totales en vitamine E étaient de 451 ppm. La BCSO saoudienne a montré une teneur élevée en acide linoléique (59,8 %). Les résultats de la CLHP soulignent que les principaux triacylglycérols (TAG) de la BCSO saoudienne étaient le 1-oleoyl-2,3-dilinoleoylglycérol (OLL; 37,7 ± 0,4 %) et le 1,2,3-

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trilinoléylglycérol (LLL; $35,9 \pm 0,3\%$). D'autre part, les TAG mineurs présents étaient le 1-palmitoyl-2,3-dilinoléylglycérol (PLL; $6,7 \pm 0,9\%$), le 1,2,3-trioléylglycérol (OOO; $6,4 \pm 0,5\%$) et le 1,2-dioléyl-3-linoléylglycérol (OOL; $5,7 \pm 1,1\%$). La BCSO saoudienne présente des propriétés physico-chimiques spécifiques et pourrait être utilisée pour des applications médicales.

Mots clés : BCSO saoudien / *p*-cymène et thymoquinone / vitamine E / acide linoléique

1 Introduction

Nigella sativa L. (Ranunculaceae), commonly known as black cumin seed and *Habat Al-barkah* in Saudi Arabia, is an annual herbaceous plant cultivated in different parts of the world. The seeds are used extensively for flavouring and medicinal purposes. Black cumin seed has been used in traditional medicine dating back to the ancient Egyptians, Greeks, and Romans. Black cumin seed (BCS) is an annual herbaceous plant cultivated in different parts of the world, mainly in countries bordering the Mediterranean Sea. It grows up to 20 to 30 cm tall, with finely divided, linear leaves. The flowers are delicate, and usually colored pale blue and white, usually with 5 to 10 petals. The fruit is a large and inflated capsule composed of 3 to 7 united follicles, each containing numerous seeds (Ismail *et al.*, 2008).

In recent times, considerable research interest has been devoted worldwide to the investigation of the black seeds for their historically alleged medicinal properties. Black cumin seeds can be used in the preparation of a traditional sweet dish and eaten with honey and syrup and for sprinkling on bread, flavoring of foods, especially bakery products and cheese (Takruri and Dameh, 1998). They also can be used in traditional medicine as a natural remedy for several illnesses that include asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, fever, dizziness and influenza, and as a carminative, diuretic, lactagogue and vermifuge (Hosseini *et al.*, 2007; Allahghadri *et al.*, 2010).

The black cumin seed oil (BCSO) is popularly used in certain cases of asthma and eczema (Ali and Blunden, 2003; Atta, 2003). BCSO is light amber in color with a characteristic herbaceous aroma. Today, BCSO is known for its nutritive properties, and is used throughout the nutritional supplement and personal care industries for various applications. Black cumin seed does in fact have remarkable healing and health properties that make it one of the most powerful medicinal plants known to man. This remarkable oil contains over 100 chemical compounds that have been analyzed by many researchers (Bertrand and Mehmet, 2011).

There are many studies have been done to show the physicochemical properties of BCSO. The BCSO properties are affected by the geographical locations in which the black cumin seed grows. It is reported that the range of percentage of free fatty acids (FFAs) of black cumin seeds is at 18.6% to 22.7% (Salma *et al.*, 2007). The range shows that different types of oils have different percentages of FFAs, based on the storage conditions and the geographical locations. The iodine value of BCSO reported by Muhammad *et al.* (2009) was 101 to 119 mg/100 g. The high iodine value of BCSO is due to the presence of large amounts of unsaturated fatty acids such as oleic and linoleic acid (Salma *et al.*, 2007). The saponification value of BCSO for a sample from Pakistan was reported by

Muhammad *et al.* (2009) as 172.6. In addition, Salma *et al.* (2007) reported that the saponification value of BCSO for a sample in Iran was 218, and a sample for Tunisia was 211. Moderate saponification value may indicate that BCSO possesses TAGs with an average molecule weight for mixtures of long chain acyl groups (predominantly of C₁₈ groups) (Gunstone, 2004).

Few studies have investigated the content of triacylglycerols (TAG) and fatty acids of the BCSO, in which complexity in the compounds was varied based on the extraction techniques and place where the seeds were grown (Gharby *et al.*, 2015). Reports examined the constituents of BCSO in addition to identifying many important compounds, such as water-soluble vitamins and minerals, phenolic compounds and essential fatty acids (Kinki, 2020), fat-soluble bio-active compounds such as sterols and tocopherols, phytosterols, polyphenols and tocopherols (Benkaci-Ali *et al.*, 2012) were also have been published. Moreover, Rao *et al.* (2007) have reported bio-active compounds such as *p*-cymene, monoterpenes, thujene, octenol with substantial amounts of flavouring agents including cineole and thymoquinone.

In general, BCSO demonstrated many pharmacological activities such as antiparasitic, antihypertensive, analgesic, antineoplastic, antibacterial properties against hepatotoxicity and nephrotoxicity due to the presence of many active components (Ketenoglu *et al.*, 2020). However, the comprehensive data on *Nigella sativa* from Saudi have been limited. Ramadan and Mörsel (2002) have studied some aspects of the physicochemical properties of *Nigella sativa* only from the center and south of Saudi. In the current study is aimed to examine and study the physicochemical properties of Saudi BCSO obtained from Qasim region.

2 Materials and method

2.1 Materials

All materials and chemicals used in this study such as methanol, chloroform, *n*-hexane, ethanol and diethyl ether, acetonitrile were analytical reagent and were used without further purification. The chemicals used for high performance liquid chromatography (HPLC) were analytical HPLC grade.

2.2 Oil extraction

Black cumin seed (BCS) was purchased from herbal market in Qasim city which is situated in the East of Saudi Arabia. The seeds were dried at 100–105 °C for 30 minutes. BCS was ground in an electrical mill to a fine powder. About 500 g powder BCS was weighed separately and placed in a thimble. The thimble was then placed in the Soxhlet reflux chamber, which was suspended above a boiling flask

containing 2500 mL hexane. The hexane which has boiling point at 60 °C was used for the extraction solvent. During the reflux, the chamber containing the milled BCS was slowly filled with hot hexane, until the hot hexane exceeded a certain temperature level and eventually overflowed and spilled over back into the boiling flask. This cycle was repeated for about 8 hours. After the extraction, the hexane was evaporated by a rotary evaporator using water bath at 70 °C and extracted BCSO was kept overnight in an oven temperature of 65 °C until no trace of solvent and further stored in the room temperature prior to the chemical analysis.

2.3 Physicochemical characteristics analysis methods

2.3.1 Acid (% FFA), iodine, saponification values and unsaponification matter

All of the above parameters were determined according to A.O.C.S. Ca 6a-40 (1989) and [Salimon *et al.* \(2006\)](#)

2.3.2 Volatile compounds

The BCS oil adheres strongly to the plant materials so that steam heat must be used to separate them by using the steam distillation method. In the distillation process, steam was passed over the ground seeds and the volatile compound evaporates off together with the steam from the plant materials. As the steam cools down, it turns back into water which was collected in a collecting vessel, and the volatile compound settles on top of this. Being lighter than water, the volatile compound can be either skimmed off from the surface of the water, or can be very easily tapped off. The content of volatile compounds were measured by gas chromatography-mass spectrometry (GC-MS). GC-MS was carried out using an Agilent 7890A GC equipped with the mass spectrometer (Agilent 5975C Inert MSD, with Triple-Axis Detector).

2.3.3 Vitamin E and fatty acids (FA) composition

The concentration of vitamin E in oils was determined by HPLC (Hewlett Packard HP1100, FLD). The analysis was performed using YMC column 150 × 6 mm I.D. The mobile phase used was composed of 0.5% isopropylalcohol/hexane and the flow rate was 1 mL/min. Total runtime for each standard and sample was 40 minutes. The injection volume was 20 µL. Detection was performed using a fluorescence detector at excitation wavelength 295 nm and emission 330 nm. Identification of tocopherol compounds was done by comparing with standard tocopherol compounds. All standards compounds were obtained from the Malaysian Palm Oil Board (MPOB). The standard concentration was 40 ppm for each component. Quantification of vitamin E was done by using the following formula:

$$xppm = \frac{V_s}{W_s} \times \frac{A_s}{A_{std}} \times \frac{V_{Istd}}{V_I} \times C_{std},$$

where:

V_s : volume of sample; W_s : weight of sample; A_s : area of sample; A_{std} : area of standard; V_{Istd} : volume of standard injected; V_I : volume of sample injected; C_{std} : concentration of standard.

The fatty acids composition was determined by conversion of oil to fatty acid methyl esters (FAME) using two step methods: acid catalyzed and base catalyzed preparations. In acid catalyzed preparation (esterification), a reagent mixture of 10 mL methanol and 2.5 mL concentrated hydrochloric acid (37%) was used. 2 g of the oil sample was placed in a small (50 mL) two-neck round-bottom flask, equipped with a standard taper joint (19/38) and short condenser. About 7.5 mL methanol was added to 1.5 mL of the previous reagent followed by 1.5 mL of toluene. The mixture was then heated at 65 °C for 1.5 hour. The heated mixture was subsequently transferred into a separating funnel. 15 mL of hexane and 10 mL distilled water were added to the mixture. The mixture was left to stand until two distinct layers emerged. The upper layer was decanted and dried using anhydrous sodium sulphate Na_2SO_4 overnight. Then 1 µL of the FAME was then injected into a gas chromatography. For base catalyzed preparation (transesterification), the FAME were prepared by adding 1 mL of *n*-hexane into 0.1 mL oil followed by 1 mL of 0.78 N sodium methoxide according to [Salimon *et al.* \(2006\)](#). The solution stirred vigorously using vortex stirrer for 10 seconds and allowed to settle for 10 minutes to separate out the clear solution of FAME from the cloudy aqueous layer. The top layer of FAME was collected carefully and analysed using gas chromatography (Model 5890 SERIES II GC, HEWLETT PACKARD, USA) software equipped with flame ionization detector (FID) and a BPX-70 fused silica capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness). The injector was maintained at 280 °C. Operating conditions were as follows: helium as the carrier gas was at a flow rate of 1 mL/min, injection volume 1 µL and a split ratio of 60:1. The oven temperature was maintained at 120 °C and increased to 245 °C and hold for 15 min at a rate of 3 °C per minute for 56.6 mins of analysis. The fatty acid methyl esters peaks were classified and quantified by comparison their peaks area and retention times with that pure standard FAME ([Bahadi *et al.*, 2019](#)).

2.4 Triacylglycerol composition

TAGs of oils were determined by using high performance sliquid chromatography (HPLC-Ultimate 3000 DIONEX) equipped with evaporative light scattering (ELS) detector and an auto-injection. The TAGs of oil were separated using commercially packed C_{18} column 5 µm × 120 Å (4.6 × 250 mm) at room temperature. The parameters of HPLC were carried out according to [Salimon *et al.* \(2006\)](#). The mobile phase was a mixture of acetone: acetonitrile (63.5:36.5) set at a flow rate of 1 mL/min. Sample preparation involved 0.1 mL sample dilution with 1.5 mL acetone: acetonitrile (63.5:36.5) mixture before putting it into HPLC and auto-injection with the total running time of 30 minutes. TAG peaks were identified based on the retention time of available commercial TAGs standard. The relative composition percentages of TAG peaks were evaluated from all peaks appeared after 15 minutes (retention time of the first TAG peak appeared).

2.5 Functional group determination

Fourier Transform Infrared Spectroscopy (FTIR) was used to identify the functional groups exist in oil and possible

Table 1. Physiochemical characteristics Saudi BCSO.

Properties	BCSO
Acid value (mg KOH/g)	14.3 ± 0.3
% FFAs	7.2 ± 0.5
Iodine value (mg/100 g)	122.7 ± 2.3
Saponification value (mg/g)	188.9 ± 6.8
Unsaponifiable matter (%)	4.8 ± 0.2

impurities exist in the oil. A very thin film of oil was covered on NaCl cells- 25 mm diameter × 4 mm thickness and the spectrum was recorded by Perkin Elmer (Spectrum GX) spectrophotometer in the range of 400–4000 cm⁻¹.

3 Results and discussion

3.1 Oil extraction

Saudi BCSO has been extracted from black cumin seeds using *n*-hexane solvent. The percentage of extracted oil was 43.7%. This is high percentage of extracted oil compared to oil content of Tunisian black cumin seeds (28.5%) (Salma *et al.*, 2007) and black cumin seeds from Turkey (28.0% to 36.44%) (Matthaus and Ozcan, 2011). The oil content of the studied black cumin seeds samples is in agreement to the Iranian black cumin seeds oil of 40.4% (Salma *et al.*, 2007). The differences between the values of BCSO content may be related to the variations of cultivated regions, storage conditions, temperatures and maturity stages. It may also be due to geographical and climatic differences where the *Nigella* seeds had been grown (Atta, 2003).

Fieldsend and Morison (2000) showed that oil and the linolenic acid content of seeds can be strongly influenced by the climatic conditions prevailing during seed growth. It is shown that the use of improved cultivars can reduce the risk of producing low-quality seeds (Fieldsend and Morison, 2000). The good cultivation for BCS production is when the climate is cool and humid, and the seeds are set in the ground correctly. Additionally, sandy loam rich in microbial activity is the most suitable soil for this type of cultivation (Animesh *et al.*, 2012). The BCS of the studies plant samples has shown considerably high oil content, as was revealed by the yield (43.7%). Thus, it can be a good raw material source for the production of many oil based products such as soap, shampoo as well as for medicinal purposes.

3.2 Physiochemical characteristics

Table 1 presents the physical properties of Saudi black cumin seed oil (Saudi BCSO) such as the acid value, free fatty acids, iodine value, saponification value, and unsaponifiable matters.

Both acid value and FFAs percentage were calculated as free oleic acid percentage content for fats and oils. Acid value (AV) is an important indicator of oil quality. AV is expressed as the amount of KOH (in milligrams) necessary to neutralize FFAs contained in one gram of oil. FFAs results were expressed in terms of acid value by multiplying the FFAs

percentage by 1.99. FFAs are an important oil quality indicator during each stage of when processing the oil. It is a measure of degumming and neutralization efficiency and a process control tool for other processes (Gunstone *et al.*, 2007).

As shown in Table 1, the AV of Saudi BCSO was 14.31 mg KOH/g. The high acid value is an indication of oil has been hydrolysed predominantly involved the enzymatic hydrolysed process. The BCSO TAGs molecule hydrolysed generating of glycerol, free fatty acids (FFAs) of BCSO essential fatty acids, diacylglycerols and monoacylglycerols as by products (Tawde *et al.*, 2013). FFAs are fatty acids exist in oil molecules or TAGs that have been hydrolyzed. Their presence indicates that degradation may occurred in the oil through poor handling during seeds harvesting or oil processing. FFAs can influence the organoleptic value of the oil. If the %FFAs are high (over 0.8%), it indicates that there has been fruit damage (frost, bruising, etc.), delays between harvest and processing or harvesting of over-ripe fruits. Low levels of FFAs value indicates that the oil was in good kept without hydrolysis (Tawde *et al.*, 2013). The result showed that Saudi BCSO consist considerably high FFAs and in agreement with other reports (Gharby *et al.*, 2015). However higher FFAs value (>19.28%) if methanol has been used for extraction solvent. This is due to highly polar solvents induce TAGs hydrolysis and saponification reactions that facilitate FFAs formation in vegetable oils (Gharby *et al.*, 2015). For the skin treatment products usage the oil with has low FFA value should be chosen to make sure the perfect oil for treatment from the skin irritation effect.

The iodine value (IV) is the number of grams of iodine consumed by 100 g of fat. A higher iodine value indicates a higher degree of unsaturation (Marina *et al.*, 2009). As can be seen in Table 1, the iodine value of Saudi BCSO was 122.7 mg/100 g. Iodine value is not the best index for oil stability since it does not take into account the positions of the double bonds available for oxidation. However, it is still important in assessing the stability of oil in oleochemical applications such as making soap. The IV value of Saudi BCSO samples were within the reported range (Kiralan *et al.*, 2014).

Saponification value, a measure of the alkali-reactive groups in oils, which can be used to predict the type of TAGs in the sample. Saponification value is a useful screening tests for characterizing types of acyl groups present in oils. As shown in Table 1, the saponification value of Saudi BCSO was 188.9 mg/100 g. In general, the saponification values for Saudi BCSO samples were ranged (188.0–190.7) and agreed with other study. The higher SV values suggesting the presence of high TAGs content (Mohammed *et al.*, 2016). However, the saponification value of the Saudi BCSO samples was higher when compared to the saponification value of the BCSO samples studied by Muhammad *et al.* (2009), which was 172.6 mg/100 g, but lower than the saponification value of the BCSO samples studied by Salma *et al.* (2007), which was 211 mg/100 g. The unsaponifiable matter on the other hands is important in determining the total quantity of the substances present in oil or fat after saponification with an alkaline hydroxide is insoluble in water but soluble in the solvent used for determination. It includes hydrocarbons, higher aliphatic alcohols, sterols (tocopherols/tocotrienols) and pigments as well as any foreign organic matter non-volatile at 103 °C (*e.g.* mineral oil or added antioxidants), which may be present

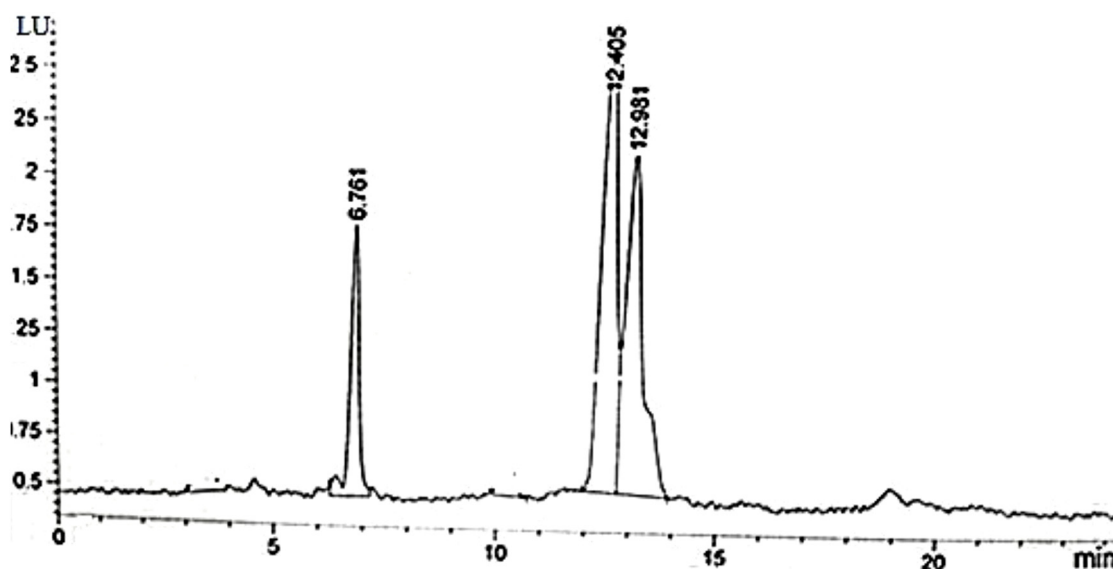
Table 2. Saudi BCSO volatile compounds percentage (%).

Name	Percentage (%)
α -Thujene	8.83 \pm 0.27
β -Pinene	1.88 \pm 0.21
<i>p</i> -Cymene	31.50 \pm 0.51
γ -Terpinene	0.88 \pm 0.04
Terpinolene	2.85 \pm 0.16
4-Terpineol	0.38 \pm 0.08
Thymoquinone	25.35 \pm 0.37
Carvacrol	4.43 \pm 0.41
3,5-Dimethylcyclohexanol	11.40 \pm 0.16
Longifolene	3.03 \pm 0.11
Paeonol	4.48 \pm 0.16
Minor compounds	4.90 \pm 0.20

Table 3. Concentration of vitamin E in Saudi BCSO.

Vitamin E	Concentration (ppm)
α -T	67 \pm 2
γ -T	195 \pm 5
γ -T ₃	189 \pm 4
Total	451 \pm 6

α -T: α -tocopherol; γ -T: γ -tocopherol; γ -T₃: γ -tocotrienols.

**Fig. 1.** HPLC chromatogram of vitamin E of Saudi BCSO.

Salimon *et al.* (2006). The unsaponifiable matter of Saudi BCSO was 4.78%. The unsaponifiable content of the studied Saudi BCSO was higher when compared to the BCSO studied by Salma *et al.* (2007), who have reported a total unsaponifiable content of about 1.49% of oil for BCSO of Iranian origin and 1.56% of oil for BCSO of Tunisian origin. Additionally, the unsaponifiable content of Pakistani BCSO was 1.8%. The different values of unsaponifiable matter in the oils from different origins is due to the difference in oil-soluble compounds content which reflects the soil and climate conditions where the samples were collected.

Table 2 shows the values of volatile compounds of Saudi BCSO. As can be seen, the main volatile compounds of Saudi BCSO are *p*-cymene (31.50%) and thymoquinone (25.35%). The levels of other volatile compounds such as 3,5-dimethylcyclohexanol, α -thujene, carvacrol, paeonol and longifolene are 11.40%, 8.83%, 4.43%, 4.48 % and 3.03%, respectively. These results are in agreement with the results from Muhammad *et al.* (2009), who has shown the content of

volatile compounds of BCSO as follows: thymoquinone (23.25%), *p*-cymene (32.02%), carvacrol (10.38%), and α -thujene (2.40%). Due to the high level of volatile compounds mentioned above, the Saudi BCSO has a typical therapeutic properties (Wallace, 2012).

Vitamin E (also known as tocopherol or alpha-tocopherol) is an antioxidant. It may help protect body cells from damage. This essential nutrient occurs naturally in many foods. It is also available as a dietary supplement. Sometimes, it's in cooperated in processed foods. Vitamin E is fat-soluble, this means the body stores and uses it as needed. It helps body nerves and muscles work well, prevents blood clots, and boosts the immune system (Kosari *et al.*, 2010). Tocopherols (T) are the most abundant form of vitamin E in the body, consisting of four different forms, which are alpha-, beta-, gamma-, and delta-tocopherol. Tocotrienols (T₃), which are found in the body to a lesser extent, also exist in four different forms, which are alpha-, beta-, gamma-, and delta-tocotrienol. All T and T₃ are available from an average diet. Every forms of vitamin E

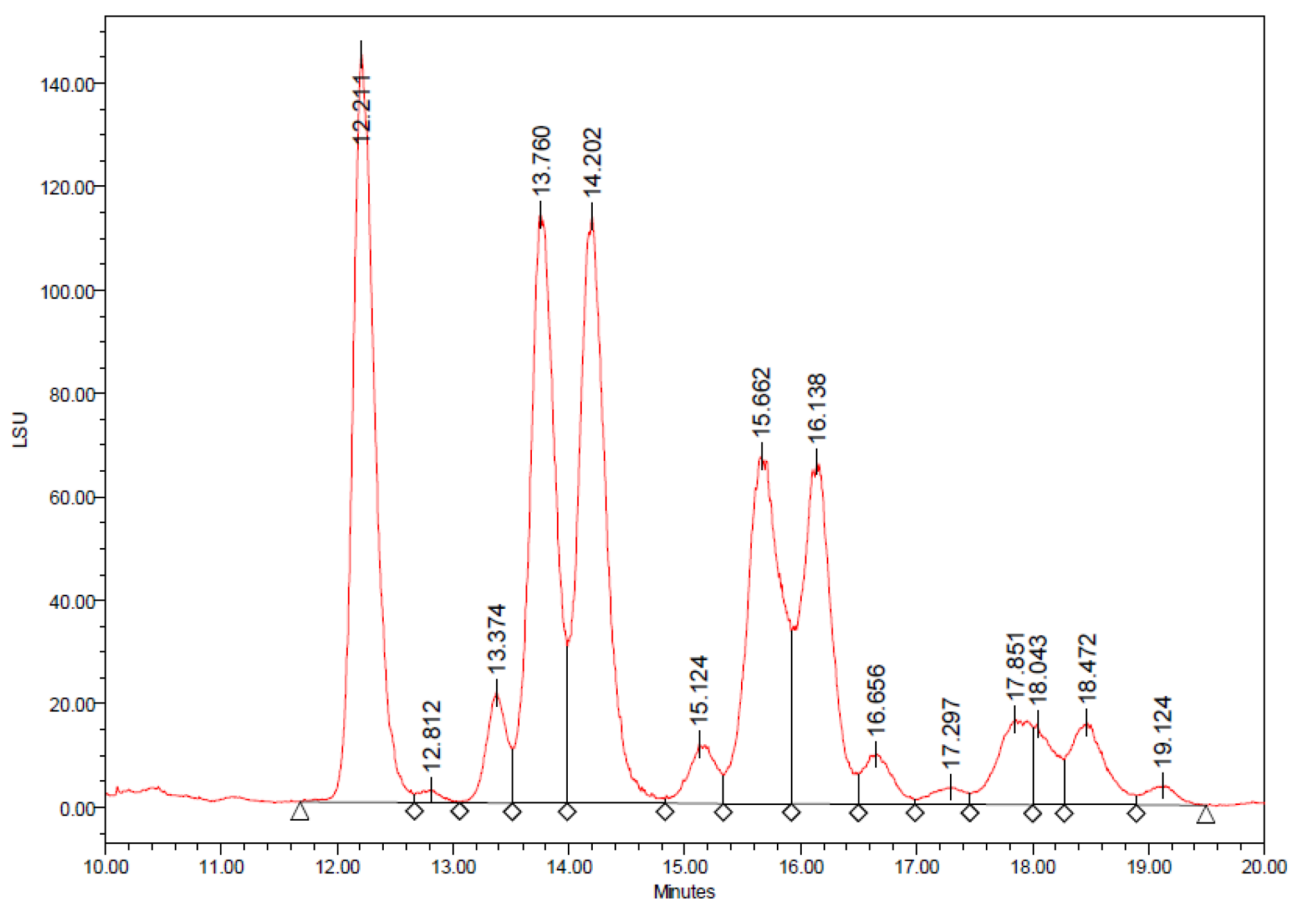
Table 4. Fatty acid composition of Saudi BCSO.

Fatty acids	Composition (%)
Myristic (C _{14:0})	0.24 ± 0.10
Palmitic acid (C _{16:0})	11.10 ± 0.20
Palmitolic acid (C _{16:1})	0.23 ± 0.12
Heptadecanoic acid (C _{17:0})	0.56 ± 0.21
Stearic acid (C _{18:0})	2.60 ± 0.41
Oleic acid (C _{18:1})	24.6 ± 1.11
Linoleic acid (C _{18:2})	58.8 ± 2.04
Arachidic acid (C _{20:0})	0.22 ± 0.02
Linolenic acid (C _{18:3})	0.4 ± 0.12
Eicosenoic acid (C _{20:1})	0.18 ± 0.02
Eicosadienoic acid (C _{20:2})	0.22 ± 0.12
Eicosatrienoic acid (C _{20:3})	0.74 ± 0.12
Behenic acid (C _{22:2})	0.11 ± 0.02

Table 5. TAGs composition (%) of BCSO.

TAGs	ECN	Composition %
LLL	42	35.9 ± 0.3
OLL	44	37.7 ± 0.4
SLL	46	0.5 ± 0.5
PLL	44	6.7 ± 0.9
OOL	46	5.7 ± 1.1
OOO	48	6.4 ± 0.5
PPL	46	2.0 ± 0.3
POP	48	4.7 ± 1.2
SOO	50	0.4 ± 0.2

ECN: equivalent carbon number; L: linoleic acid; O: oleic acid; P: palmitic acid; S: stearic acid.

**Fig. 2.** HPLC chromatogram of TAGs of Saudi BCSO.

splay an important role to reduce disease. Studies have proved that α -T and γ -T₃ decrease inflammatory prostaglandin synthesis, as well as limit inflammatory responses to lipid hydroperoxide exposure (Shibata *et al.*, 2010). Three forms of vitamin E (α -T, γ -T and γ -T₃) have been found in Saudi

BCSO with different proportions. The results are shown in Table 3.

The Saudi BCSO; α -T, γ -T and γ -T₃, which are shown in Figure 1. The chromatogram illustrated α -T peak at 6.76 min, γ -T peak at 12.4 min and γ -T₃ peak at 12.98 min respectively.

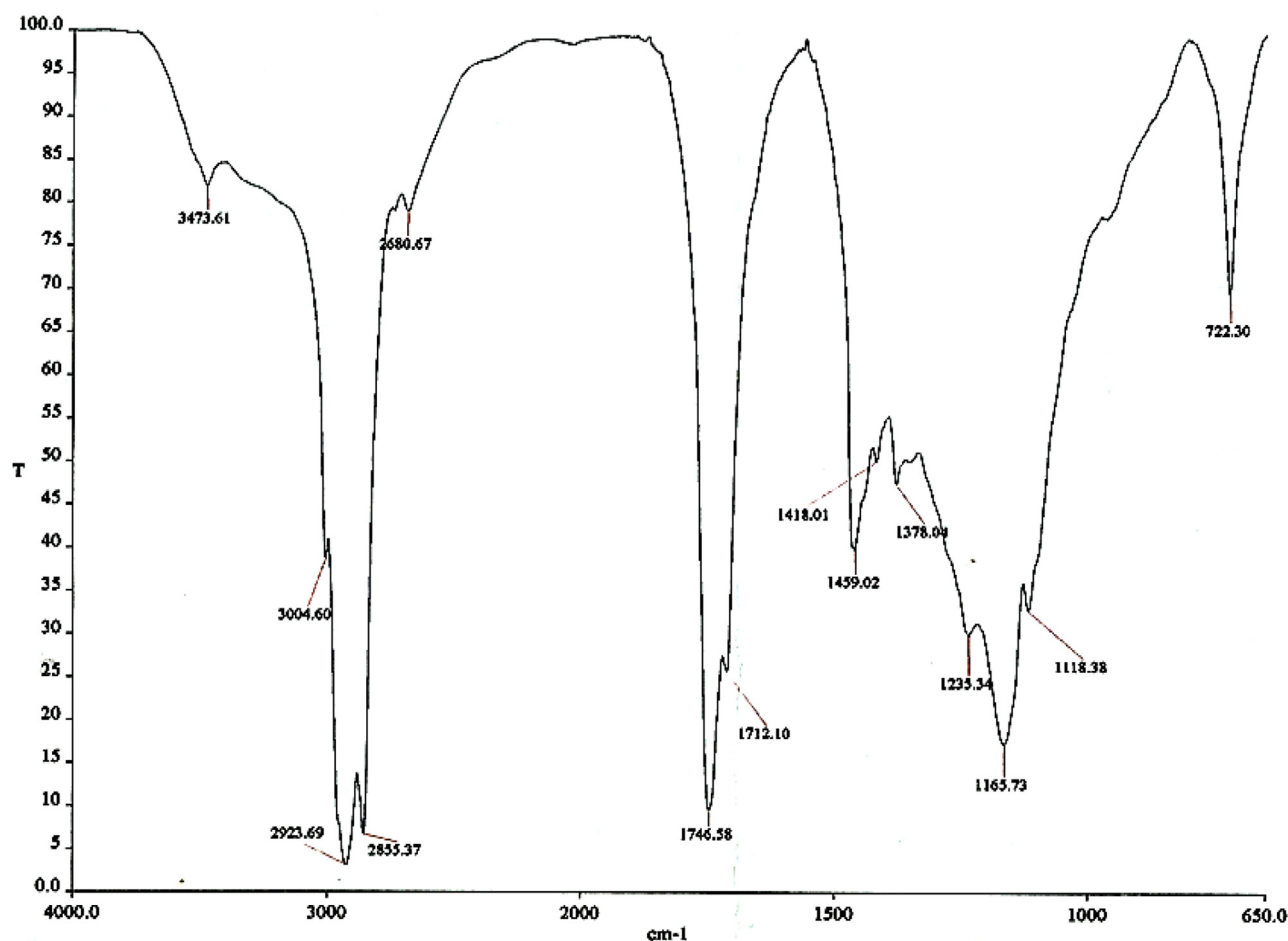


Fig. 3. FTIR spectrum of Saudi BCSO.

The peaks showed that, α -T concentration was at 67 ppm, γ -T concentration was at 195 ppm and γ -T₃ concentration was at 189 ppm. Therefore, Saudi BCSO had relatively high value of γ -T and γ -T₃.

3.3 Fatty acid composition

Fatty acid compositions of Saudi BCSO is shown in Table 4. The major fatty acids were linoleic (59.8%), oleic (25.6%) and palmitic (11.1%) acids. The fatty acid compositions of the studied Saudi BCSO were also comparable with other results by Muhammad *et al.* (2009) where content of palmitic acid (12.07%), oleic acid (8.7%) and linoleic acid (58.9%) and Salma *et al.* (2007) where content of palmitic acid (17.2% to 18.4%), oleic acid (23.7% to 25%) and linoleic acid (49.15% to 50.3%). It is found that the oleic and linoleic acids contents of Saudi BCSO resemble that of walnut oil, which consists of 13.8% to 26.1%, and 54.9% to 60.6% respectively. They also resemble that of soybean oil, which contains palmitic acid at 11.4%, linoleic acid at 52.1% and oleic acid at 24.7% (Xu *et al.*, 2007).

3.4 Triacylglycerols profile

The TAGs profile is characterized by reversed phase HPLC, where the mechanism in separating the TAGs involves the chain length and degree of unsaturation of the fatty acids (Gutierrez and Barron, 1995). The content of TAGs in Saudi BCSO is shown in Table 5. The results showed that Saudi BCSO was classified as high unsaturation oil with 85.7% unsaturation TAGs. The dominant TAGs peaks of Saudi BCSO were 1-oleoyl-2,3-dilinoleoylglycerol, OLL (37.7±0.4%), 1,2,3-trilinoleoylglycerol, LLL (35.9±0.3%), 1-palmitoyl-2,3-dilinoleoylglycerol, PLL (6.7±0.9%), 1,2,3-trioleoylglycerol, OOO (6.4±0.5%) and 1,2-dioleoyl-3-linoleoylglycerol, OOL (5.7±1.1%) respectively. Figure 2 represents the HPLC chromatogram of TAGs of Saudi BCSO.

3.5 Functional groups in oil

Figure 3 illustrate the IR spectrum of BCSO obtained with the Fourier transform infrared spectroscopy, FTIR transmission cell set up in the range of 400 to 4000 cm⁻¹. In general,

there are two major functional groups present in the triacylglycerol molecule of oil, the carbonyl C=O of ester or/and carboxylic and C=C groups. The result shows that the FTIR spectrum shows characteristics of strong absorption bands of BCSO at 1744 cm^{-1} and a band at 1165 cm^{-1} (ester carbonyl functional group). The peaks at $1239\text{--}1021\text{ cm}^{-1}$ referred to as --C--O--C stretching vibration for ester group. Peaks at wave numbers at 3005 cm^{-1} indicated the =C--H stretch for sp^2 (aliphatic) of unsaturation in BCSO. However, the peak at wave number of 1710 cm^{-1} was also observed. This is attributed to the carbonyl C=O of carboxylic acid functional group (--COOH). This observation is likely to be expected due to the considerable high percentage of FFAs presents in Saudi BCSO of $7.19 \pm 0.5\%$.

4 Conclusion

The results showed that the physicochemical properties of Saudi BCSO contains high percentage of unsaturation TAGs especially composing linolate acyl group, which are the essential polyunsaturated fatty acids source. Therefore, Saudi BCSO can be classified as an essential unsaturated oil. Additionally, the volatile compounds of Saudi BCSO were as good source of bioactive compounds (especially *p*-cymene, thymoquinone and vitamin E) with high concentration which support their use in traditional medicine. Moreover, the Saudi BCSO can be considered as having a good source of natural antioxidant and anti-inflammatory activities that play an important role in the prevention of many diseases.

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