Fatty Acids, Tocopherol, and Sterol Contents of Some *Nigella* Species Seed Oil

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Abstract

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The lipid compositions of the seed oils of some *Nigella* species were investigated. The total oil content of the seeds ranged from 28.0 to 36.4%. GC-MS fatty acid compositional analysis of the *Nigella* seed oils revealed the content of linoleic acid to be the highest (40.3–58.9%). Other prominent fatty acids were as follows: oleic (18.7–28.1%), palmitic (10.1–12.5%), 22:1 D11 (3.2–3.8%) and stearic (2.6–3.1%) acids. All the Nigella seed oils analysed exhibited differences in their tocopherol contents and the differences were estimated. The oils extracted from the seeds contained between 1.70–4.12 mg/100 g α-T, 0.97–4.51 mg/100 g γ-T, and 4.90–17.91 mg/100 g β-T3. The total tocopherol content in seeds varied between 9.15 mg/100 g to 24.65 mg/100 g. The compositions of the sterol fractions were determined by gas liquid chromatography. The total amounts of sterols ranged between 1993.07 mg/kg to 2182.17 mg/kg. The main component was β-sitosterol (48.35–51.92%), followed by 5-avenasterol, campesterol, and stigmasterol.

Keywords: black cumin; seed oil; *Nigella* spp.; fatty acids; tocopherol; sterols

Black cumin (*Nigella sativa* L.) belongs to Ranunculaceae family and is native to some parts of the Mediterranean region. It is known as "çörek otu" in Turkish. Its seeds are not related to cumin or caraway, both of which belong to Apiaceae family. It grows in the mediterranean countries to a maximum height of about 60 cm and is cultivated in Turkey (Babayan *et al.* 1978; Başoğlu & Bayrak 1984; Türker 1996; Ramadan & Mörsel 2002). Black cumin seeds are used as spice in bakery products and other food applications. Also, its oil is used as edible oil (Aitzetmuller *et al.* 1997). Oilseeds are important sources of oils being of nutritional, industrial and pharmaceutical importance. Non-conventional oilseeds are under consideration because their constituents have unique chemical properties and may augment the supply of edible oils (Cherry & Kramer 1989; Ramadan & Mörsel 2002; Ramadan & Mörsel 2003). The spicy seeds of this plant have proclaimed medicinal use dating back to the ancient Egyptians, Greeks, and Romans. In recent times, considerable research interest has been devoted world wide to the investigation of the black seeds for their historically alleged medicinal properties. The black seed oil is popularly used in certain cases of chronic cough and as diuretic or carminative agents and bronchial asthma (Zawahry 1963; Baytop 1984; Khan 1999).

In consideration of the potential utilisation, detailed knowledge of the composition of Nigella sativa seed oil is of major importance. Little infor-

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mation is known concerning the exact composition of *N.sativa* seed oils (Başoğlu & Bayrak 1984; Üstün *et al.* 1990). In one study (Aitzetmuller *et al.* 1997), it was mentioned that *Nigella sativa* seed oil samples of Turkish source contained 57.2% linoleic, 22.0% oleic, and 11.8% palmitic acids as major fatty acids. The sterols from Nigella sativa oil have been isolated and characterised as cholesterol, campesterol, campestanol, stigmasterol, sitosterol, stigmastanol, Δ5-avenasterol, Δ7-stigmasterol, Δ7-avenasterol (Salama 1973; Menounos *et al.* 1986). The fatty acid composition of *Nigella sativa* seeds of Turkish origin has been investigated (Başoğlu & Bayrak 1984; Şener *et al.* 1985; Nergiz & Ötleş 1993; Türker 1996; Aitzetmuller *et al.* 1997) and found to contain a specific pattern of certain fatty acids.

There exist limited studies on the oil properties and the contents of fatty asids, sterols, and tocopherols of black cumin oil. Khan (1999) reviewed the chemical and medicinal properties of *Nigella sativa* seeds. Saleh Al-Jasser (1992) reported in his study on the black cumin seeds growing in Saudi Arabia stating that linoleic and oleic acids were the major unsaturated fatty acids while palmitic acid was the main saturated one.

The present study is a preliminary investigation of oil content the fatty acid only not clear tocopherol content and sterols of black cumin of the potentially most useful seeds.

MATERIAL AND METHODS

Material. *Nigella* spp. seeds (black cumin) (sample 1 *N. damascene,* sample 2 to spamle 14 *N. sativa*) were obtained from Germany and Turkey. Only sample 14 was obtained from Konya in Turkey, the others having been provided from Germany. The seeds were transported to the laboratory, and subsequently cleaned in an air screen cleaner to remove all foreign matter such as dust, dirt, stones and chaff, while immature and broken seeds were discarded as well.

Reagents. Petroleum ether (40–60°C) was of analytical grade (> 98%; Merck, Darmstadt, Germany). Heptane and tert-butyl methyl ether were of HPLC grade (Merck, Darmstadt, Germany). Tocopherol and tocotrienol standard compounds were purchased from CalBiochem (Darmstadt, Germany). Betulin, β-sitosterol, campesterol, and stigmasterol were obtained from Aldrich (Munich, Germany).

Oil content. The oil content was determined according to the method ISO 659:1998. About 2 g of the seeds were ground in a ball mill and extracted with petroleum ether in a Twisselmann apparatus for 6 hours. The solvent was removed using a rotary evaporator at 40°C and 25 Torr. The oil was dried in a stream of nitrogen and stored at –20°C until used.

Fatty acid composition. The fatty acid composition was determined following the ISO standard (ISO 5509:2000). Briefly, one drop of the oil was dissolved in 1 ml of *n*-heptane, 50 µg of sodium methylate was added, and the tube was closed and agitated vigorously for 1 min at room temperature. After the addition of 100 µl of water, the tube was centrifuged at 4500 g for 10 min and the lower aqueous phase was removed. Then, 50 µl of HCl (1 mol with methyl orange) was added, the solution was shortly mixed, and the lower aqueous phase was rejected. About 20 mg of sodium hydrogen sulphate (monohydrate, extra pure; Merck, Darmstadt, Germany) was added, and after centrifugation at 4500 g for 10 min, the top n-heptane phase was transferred to a vial and injected in a Varian 5890 gas chromatograph with a capillary column, CP-Sil 88 (100 m long, 0.25 mm ID, film thickness 0.2 µm). The temperature program was as follows: from 155°C; heated to 220°C (1.5°C/min), 10 min isotherm; injector 250°C, detector 250°C; carrier gas 36 cm/s hydrogen; split ratio 1:50; detector gas 30 ml/min hydrogen; 300 ml/min air and 30 ml/min nitrogen; manual injection volume less than 1 µl. The peak areas were computed by the integration software, and the percentages of fatty acid methyl esters (FAME) were obtained as weight percent by direct internal normalisation.

Tocopherols. For the determination of tocopherols, a solution of 250 mg of oil in 25 ml of *n*-heptane was directly used for the HPLC. The HPLC analysis was conducted using a Merck-Hitachi low-pressure gradient system, fitted with a L-6000 pump, a Merck-Hitachi F-1000 fluorescence spectrophotometer (detector wavelengths for excitation 295 nm, for emission 330 nm), and a D-2500 integration system. The samples in the amount of 20 µl were injected with a Merck 655-A40 autosampler onto a Diol phase HPLC column 25 cm × 4.6 mm ID (Merck, Darmstadt, Germany) using a flow rate of 1.3 ml/min. The mobile phase used was *n*-heptane/*tert*-butyl methyl ether (99 + 1, v/v) (Balz *et al.* 1992).

Sterols. The sterol composition of the oils was determined following ISO/FIDS 12228:1999 (E).

Briefly, 250 mg of oil was saponified with a solution of ethanolic potassium hydroxide by boiling under reflux. The unsaponifiable matter was isolated by solid-phase extraction on an aluminium oxide column (Merck, Darmstadt, Germany) on which fatty acid anions were retained whille sterols passed through. The sterol fraction was separated from unsaponifiable matter by thin-layer chromatograph (Merck, Darmstadt, Germany), re-extracted from the TLC material, and afterward, the composition of the sterol fraction was determined by GLC using betulin as the internal standard. The compounds were separated on a SE 54 CB (Macherey-Nagel, Düren, Germany; 50 m long, 0.32 mm ID, 0.25 µm film thickness). Further parameters were as follows: hydrogen as carrier gas, split ratio 1:20, injection and detection temperature adjusted to 320°C, temperature program, 245°C to 260°C at 5°C/minutes. The peaks were identified either by means of standard compounds (β-sitosterol, campesterol, stigmasterol), a mixture of sterols isolated from rape seed oil (brassicasterol), or a mixture of sterols isolated from sunflower oil (Δ7-avenasterol, Δ7-stigmasterol, and Δ7-campesterol). All other sterols were identified by GC-MS at first and afterward by comparison of the retention times.

Each method was carried out in triplicate for each sample. The mean values are given in the tables, without the standard deviations, because such values would represent only the deviation of the method and not the variation of the respective sample.

RESULTS AND DISCUSSION

The total oil contents of black cumin seeds are given in Table 1. The oil content of seeds ranged from 28.0% to 36.44%, followed sample 4 (35.67%), 3 (35.38%), 7 (35.06%), and 9 (34.76%). The oil content of *N. damascene* was found, to be low compared with others. Generally, the oil contents of black cumin seeds were established to be higher compared with most oil seeds such as chakeberry (19.3%), blackcurrant (22.0%), and rosehip (8.2%). Şener *et al.* (1985) established 26.6% oil in *Nigella sativa* seed. Also, the oil contents of *Nigella sativa* seeds collected from the Kütahya, Denizli and Konya provinces were quite similar, 24.4%, 29.5%, and 29.7%, respectively (Üstün *et al.* 1990). The differences between the values of the seed oil content can be probably due to growing, climatic, and environmental conditions as well as analytical conditions and localities.

Table 1. Oil contents of black cumin seeds

Sample	Oil concentrations (%)
1 *N. damascene*	28.0
2 *N. sativa*	30.4
3 *N. sativa*	35.4
4 *N. sativa*	35.7
5*N. sativa*	36.4
6 *N. sativa*	34.1
7 *N. sativa*	35.1
8 *N. sativa*	32.8
9 *N. sativa*	34.8
10 *N. sativa*	33.0
11 *N. sativa*	33.8
12 *N. sativa*	31.2
13 *N. sativa*	32.8
14 *N. sativa*	34.7

The identification of the individual fatty acids of *Nigella sativa* seed oil samples was carried out by gas chromotography. Fatty acid compositions of the 13 investigated seed oils are given in Table 2. The results showed that the oils of all black cumin seeds used in this study had higher linoleic acid contents (between 40.26% to 58.97%) than were those of other fatty acids. On the other hand, oleic acid contents of seed oils varied from 18.72% (sample 3) to 28.14% (sample 1). Table 2 reveals that the major components of fatty acids are linoleic and oleic acids. The oils also contain appreciably larger amounts (10.11% to 12.49%) of saturated normal chain fatty acids, especially palmitic acid. In addition to the common fatty acids, measurable amounts of 14:0, 20:0, and 24:0 saturated fatty acids were detected in the investigated samples. 22:1 acid was absent from all the samples that were investigated. According to the results obtained with *Nigella* samples, 20:2 acid showed always a much larger peak than 20:1 acid. 20:1 acid was considered to be a specific chemotaxonomic criterium for *Nigella* species. This is very rare indeed with other seed oils of the plant kingdom. Aitzetmuller *et al.* (1997) established 20:1 and 20:2 acids in *Nigella* species. Therefore, it is believed that 20:2 > 20:1 could also serve as an identity criterium for authentic *N. sativa* seed oils. However, it is interesting to note that the

Table 2. Fatty acid compositions of black cumin seeds (%)

Sample	Fatty acids													
	14:0	16:0	16:1	18:0	18:1D9	18:1D11	18:2	18:3	20:0	20:1	20:2	22:1	24:0	Total
1	0.0	10.1	0.2	2.9	28.1	0.7	40.3	0.2	0.2	0.5	3.5	0.0	0.0	86.7
2	0.6	12.0	0.2	2.6	18.7	1.1	58.1	0.3	0.2	0.4	3.8	0.1	0.4	98.4
3	0.3	12.1	0.2	2.7	21.2	1.1	57.0	0.3	0.2	0.4	3.2	0.0	0.3	99.1
4	0.0	11.5	0.2	3.0	20.9	1.0	58.5	0.3	0.2	0.4	3.2	0.1	0.3	99.5
5	0.3	12.1	0.2	2.6	20.0	1.1	58.1	0.3	0.2	0.4	3.5	0.1	0.4	99.1
6	0.3	12.1	0.2	2.6	20.1	1.1	58.1	0.3	0.2	0.4	3.5	0.0	0.3	99.2
7	0.0	12.5	0.0	2.7	21.9	1.2	56.9	0.4	0.0	0.4	3.3	0.0	0.0	99.4
8	0.2	12.1	0.2	2.6	19.1	1.1	58.9	0.3	0.2	0.4	3.7	0.0	0.3	99.2
9	0.2	11.9	0.3	3.0	21.0	1.1	57.2	0.3	0.2	0.4	3.2	0.0	0.3	99.1
10	0.2	12.2	0.2	2.7	21.2	1.1	57.3	0.3	0.2	0.4	3.3	0.0	0.3	99.3
11	1.1	12.3	0.3	2.6	19.5	1.1	56.7	0.4	0.2	0.4	3.8	0.0	0.3	98.4
12	0.0	11.7	0.2	3.1	20.8	1.0	58.1	0.3	0.2	0.4	3.3	0.0	0.3	99.6
13	0.1	11.7	0.2	3.1	20.7	1.0	58.0	0.3	0.2	0.4	3.3	0.1	0.3	99.4
14	–	11.5	–	3.2	19.7	1.0	58.7	0.4	0.2	-	-	-	-	94.7

content of 20:1 Δ11*cis* acid in the *Garidella* seed oil is very high (20.7%). The results reported here are in accordance with those of Aitzetmuller *et al*. (1997) on *N. sativa*, Akcasu and Kavalali (1990) on *N. damascena*, and Nergiz and Ötleş (1993) on *N. sativa*. Akcasu and Kavalali (1990) established 4.2. 20:2 acid in *N. damascena*. In other study, Vioque *et al*. (1994) found 0.8% of 20:1 acid, but no 20:2 acid in *N. damascena*. Üstün *et al*. (1990) definitely claimed the absence of 20:2 acid in three samples of *N. sativa* seed which contained high levels of free fatty acids. It is believed that these fatty acids (20:1 and 20:2) are a chemotaxonomic characteristic of *Nigella* genus, and that this could also serve as an identity criterium fore genuine black cumin seed oil

Table 3. Tocopherol contents of black cumin seeds (mg/100 g)

Sample	Tocopherols									
	α-T	α-T3	β-T	γ-T	β-T3	P8	γ-T3	δ-T	δ-T3	Total
1	4.12	3.47	0.42	1.97	4.90	0.00	0.00	2.15	0.64	17.68
2	2.17	0.21	0.94	3.99	16.34	0.00	0.00	0.00	0.00	23.64
3	1.83	0.18	2.71	4.51	15.41	0.00	0.00	0.00	0.00	24.65
4	0.95	0.11	0.22	1.34	12.85	0.00	0.00	0.20	0.00	15.67
5	1.86	0.29	0.00	3.72	14.37	0.00	0.00	0.41	0.00	20.65
6	1.96	0.22	0.00	3.95	17.91	0.00	0.00	0.48	0.00	24.52
7	3.82	0.20	6.96	4.53	11.40	0.73	0.00	0.28	0.00	27.92
8	2.64	0.00	0.95	3.83	15.24	0.00	0.00	0.34	0.00	23.00
9	1.85	0.00	1.35	3.68	13.14	0.00	0.00	0.35	0.00	20.37
10	1.70	0.17	1.25	3.99	14.88	0.00	0.00	0.40	0.00	22.39
11	3.75	0.35	0.00	3.83	11.33	0.40	0.40	0.00	0.00	19.67
12	0.63	0.08	0.00	0.97	7.09	0.39	0.39	0.00	0.00	9.15
13	0.81	0.00	0.00	1.09	8.21	0.00	0.00	0.00	0.00	10.12
14	4.80	0.00	2.30	1.90	14.20	0.00	1.00	0.00	0.40	24.6

(Aitzetmuller *et al.* 1997). Oleic and linoleic acids contents of black cumin oil resemble those of walnut oil, (13.8% to 26.1%) and (54.9% to 60.6%), respectively (Zwarts *et al.* 1999). The high concentration of linoleic acid in black cumin seed oil is the cause of the high nutritional value of these oils as linoleic acid is one of the three essential fatty acids (Femenia *et al.* 1995).

Tocopherols are natural antioxidants with biological activity. Especially, the antioxidant effect is higher due to high γ-tocopherol content. Tocopherol contents of blackcumin seed oil are given in Table 3. All the seeds analysed were found to exhibit differences. Total tocopherol content of the seeds varied between 9.15 mg/100 g to 27.92 mg/100 g (sample 7). The tocopherol composition of all seed oils was dominated by α-T, γ-T, and β-tocotrienol. δ-Tocotrienol was found only in sample 1. Zeitonun and Neff (1995) reported 42.90 mg/kg of α-tocopherol, 26.30 mg/kg of β-tocopherol, 4.83 mg/kg of δ-tocopherol, and 118.70 mg/kg of γ-tocopherol. The main biochemical function of tocopherols is believed to reside in the protection of polyunsaturated fatty acids against peroxidation (Beringer & Donpert 1976; Kamal-Eldin & Andersson 1977).

As a result, black cumin (*N. sativa*) can be a good source of oils due to their abundance in the seeds. At the same time, the oils are rich in fatty acid, tocopherol, and sterol contents.

Sterol contents of black cumin oil are given in Table 4. The concentration of total sterols ranged from 1993.07 mg/kg to 2887.28 mg/kg. Abd Alla (1997) determined 59.10% sitosterol, 10.0% campesterol, and 16.50% stigmasterol. In other study, in both types of seed oil examined, β-stesterol (32.2–34.1% of total sterol content) represented the main component of phytosterols followed by Δ5-avenasterol (27.8–27.9% of total sterol content), and Δ7-avenasterol (18.5–22.0% of total sterol content (Ramadan & Morsel 2002). From these values, it is clear that both seed oils extracted are remarkably similar in their sterol compositions. Ramadan and Mörsel (2003) established 12.0% to 8.20% stigmasterol, 35.3% to 20.8% β-sitosterol, 9.0% to 5.0% Δ5-avenasterol, and 43.%7 to 66.0% Δ7-avenasterol for SG and ASG, respectively. In the order of decreasing prevalence, β-sitosterol > campesterol > stigmasterol and 5-avenasterol were the major sterols of black seed oils.

Table 4. Sterol contents of black cumin seeds

Sample	Sterols															Total (mg/kg)
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	
1	0.71	–*	2.23	10.32	0.61	11.56	0.48	0.29	1.00	48.35	2.47	15.93	1.25	1.85	2.96	2182.17
2	–	–	–	–	–	–	–	–	–	–	–	–	–	–		–
3	0.70	–	2.06	9.88	0.56	10.52	0.52	0.18	1.00	51.92	2.29	15.02	1.29	1.44	2.61	2155.84
4	–	–	–	–	–	–	–	–	–	–	–	–	–	–		–
5	–	–	–	–	–	–	–	–	–	–	–	–	–	–		–
6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7	0.87	–	2.29	10.65	0.54	11.36	0.40	0.21	0.97	50.01	2.38	15.46	1.05	1.11	2.71	2086.16
8	0.65	–	2.36	10.58	0.57	9.95	0.35	–	0.99	49.99	2.50	16.60	1.20	1.27	2.99	1993.07
9	–	–	–	–	–	–	–	–	–	–	–	–	–	–		–
10	–	–	–	–	–	–	–	–	–	–	–	–	–	–		–
11	–	–	–	–	–	–	–	–	–	–	–	–	–	–		–
12	–	–	–	–	–	–	–	–	–	–	–	–	–	–		–
13	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
14	1.25	–	1.21	14.06	0.94	18.38	0.72	–	1.16	46.68	2.78	7.35	1.24	2.39	1.83	2887.28

A – cholesterol; B – brassicasterol, C – 24-methylen-cholesterol; D – campesterol, E – campestanol; F – stigmasterol; G – 7-camersterol; H – 5,23-stigmastadienol; I – chlerosterol; J – B-sitosterol; K – sitostanol; L – 5-avenasterol; M – 5,24-stigmastadienol; N – 7-avenastenol; O – 7-avenastenol

*nonidentified

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