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Some characteristics of nigella (*Nigella sativa L.*) seed cultivated in Egypt and its lipid profile

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Abstract

Physico-chemical properties of nigella seeds cultivated in Egypt were determined. Physical and chemical analyses of crude oils extracted from the seeds, by two different methods were also performed. The results revealed that nigella seed is a good source for oil and protein. Percentage of oil extraction, as well as physical and chemical properties of the crude oil, was influenced by the extraction methods. TLC and GLC analysis indicated that nigella seed oil contained significant amounts of sterols. Linoleic ($C_{18:2}$), oleic ($C_{18:1}$) and palmitic ($C_{16:0}$), as in most of the common edible oils, are the main fatty acids. The oil was rich in β-sitosterol that inhibits the absorption of dietary cholesterol.

Keywords: Nigella; Fatty acid composition; Sterols; Physical properties

1. Introduction

Nigella (*Nigella sativa L*) is an annual herbaceous plant belonging to the Ranuculacea family. Mature seeds are consumed for edible and medical purposes. The seeds are used as seasoning for vegetables, legumes and different types of baked products (Atta & Imaizumi, 1998; Üstun, Kent, Gekin, & Clvelekoglu, 1990). In Egyptian folk medicine, nigella seeds are used as carminatives, diuretics, and for delayed menses and lactation, while their oil has protective action against histamine induced bronchospasm, cough and bronchal asthma (Mahfouz & El-Dakhakhny, 1960; Soliman, 1978; Tappozada, Mazloum, & El-Dakhakhny, 1965). Proximate analysis of whole mature nigella seeds showed that the moisture content ranged from 5.52 to 7.43%, crude protein from 20 to 27%, ash from 3.77 to 4.92%, ether-extractable lipid from 34.49 to 38.72% and carbohydrates from 23.5 to 33.2% (Abdel-Aal & Attia; 1993, Salem, 2001; Takruri & Dameh, 1998). Fixed oil of nigella seeds is rich in linoleic, oleic and plamitic acids (Abdel-Aal & Attia, 1993; Babayan, Koottungal, & Halaby, 1978; Gad, El-Dakhakhny, & Hassan, 1963). Several authors have investigated the essential oil of nigella seeds and isolated and identified active constituents that have beneficial clinical effects (Gad et al., 1963; Karawya et al., 1994; Mahfouz & El-Dakhakhnay, 1960). Egyptians believe that nigella seeds increase human immunity. The oil has been produced by pressing the raw or roasted seeds. The pressed cake (by-product) has not been efficiently utilized, since it can not be used directly for animal feed. Therefore, other possibilities for nigella seed cake application have been investigated by Atta and Imaizumi (1998). They found that an ethanolic extract from defatted nigella seeds had an equivalent effect to that of TBHQ, but it is not yet at a stage to be incorporated in an industrial process.

The present work generates special data on the characteristics of nigella seeds cultivated in Egypt and their oil properties, including lipid classes, fatty acid composition and sterol contents of the extracted oil, obtained by two different methods (cold press and solvent extraction).

2. Materials and methods

2.1. Materials

Mature nigella (*Nigella sativa* L.) seeds were supplied by the Medicinal and Aromatic Research Department, Horticulture Research Station, Agriculture Research Center, Alexandria, Egypt. Standard fatty acid methyl

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esters were purchased from Sapelco (Sigma-Aldrich, Japan K. K. Shuo Ku, Tokyo 103). Sterols were obtained from Sigma Chemical Co. (St. Louis, MO) while all other chemicals used were AR grade.

2.2. Methods

2.2.1. Physical properties of nigella seeds

Seed index (weight of 1000 seeds in grammes), seed volume (volume of 1000 seeds in cm^3) and bulk density (g/cm^3) of nigella seeds were estimated according to the method of Kramer and Twigg (1962).

2.2.2. Crude oil extraction

Nigella seeds were cleaned and crushed using an electric grinder (National, model MX-915 C Japan) at speed 6 for 2 min and then passed through a 35 mm (42 mesh) sieve. The milled seeds were divided into two portions. The first one was subjected, immediately, to a laboratory-type hydraulic press (Carver model 27595/N2759-FREDS Carver Inc. Wabash, USA) using a pressure of 11 kg/cm^2 for 1 h to obtain the oil, which was filtered through a glass funnel plugged with cotton. The second portion of ground seeds was used to extract the oil by petroleum ether (40–60 °C) using a Soxhlet apparatus, as described by AOAC (1990). The oil was transferred into glass sealed amber dark bottles. The solvent residue in the oil was removed under a stream of nitrogen, capped and stored at −18 °C until analysed.

2.2.3. Determination of physical and chemical properties of crude oil

Specific gravity (SG), melting point (MP) (determined using micro melting point apparatus) (Yanagimoto, Seisakusho, Japan), refractive index (RI) measured using Abeé Refractometer at 20 °C, and colour estimated, using a Lovibond (Lovibond R-3 Field Comparator, serial no. 3F 1547, England) of the crude oil were determined following the methods of AOAC (1990).

Free fatty acid content (FFA; as % oleic acid), iodine value (IV; Wijs), peroxide value (PV; meq O_2/kg oil), saponification number (SN) and percentage of unsaponifiable matter (USM%) of crude oils were determined according to AOAC (1990). The crude oil was fractionated on a 200 × 200 × 0.25 mm precoated silica gel glass plate (Merck, Darmstadt, Germany) according to the method of Mangold (1969). Lipid fractions were identified by comparison of their R_F values with the authentic standards. Thin-layer chromatograms were sprayed with Charing reagent (Rouser, Fieskher, & Yamamota 1970), scanned at 700nm and the percentage of each fraction calculated.

2.2.4. Determination of fatty acid composition

Lipid fractions of cold pressed crude oil, separated by TLC, were individually dissolved in known amounts of *n*-hexane. For analysis of fatty acids of the crude oils and for each individual fraction, pentadecanoic ($C_{15:0}$) was included as internal standard. Fatty acids were esterified and analysed by Shimadzu GC 14A Gas Chromatography on a 30-metre (0.32 mm) glass capillary column coated with OMEGA Wax 320 equipped with FID detector. Identification of fatty acid methyl esters was based on comparison of retention times of unknown peaks with authentic fatty acid methyl esters. The quantity of each fatty acid methyl ester was estimated by comparison with known amounts of pentadecanoic methyl ester. Fatty acid composition was expressed as weight percent (%) of total fatty acid methyl ester.

2.2.5. Determination of sterols

Approximately 300 nmol (251 μg) of 5-α-cholestane in chloroform, as internal standard, was added to crude oil (ca. 5–10 mg) in a glass-stoppered test tube, and solvents removed under nitrogen gas. Saponification and transesterification of unsaponifiable matter were carried out according to Kamal-Eldin, Frank, Razdan, Tengblad, and Vessby (2000). Sterols were analysed by Shimadzu Gas Chromatography GC-4 CNPF using a 3 mm × 2 m stainless steel column packed with Gas Chrom Q 60–80 mesh as support, coated with 3% Silicone OV-17 and equipped with FID using air and helium at 1.8 and 0.6 kg/cm^2, respectively. The carrier gas was nitrogen at 1.8 kg/cm^2. Injection and detection temperatures were 300 °C and column temperature was 260–270 °C.

2.2.6. Statistical analysis

Values represented means and standard deviations for three replicates. Statistical analysis was carried out by student's *t*-test according to Fisher (1970).

3. Results and discussion

3.1. Physical and chemical properties of Nigella seeds

The physical properties of seeds determine how they are handled during manufacture (Juliano, Perez, & Ard, 1990). Mean values of seed index and volume of 1000 seeds, as well as their bulk density, were 2.21 g, 2.76 ml and 0.80 g/cm^3, respectively (Table 1). This is important from the oilseed view, as the greater the seed weight is, the higher the oil content (Basha, 1994; Mohamed, 1996). The estimated values for both seed index and volume were lower than those reported by Abdel-Aal and Attia (1993); therefore their bulk density (0.80 g/cm^3) was higher than that mentioned (0.79 g/cm^3) in the literature. These variations could be explained by the diversity in maturity and phenotype of the investigated seeds.

Table 1
Some characteristics of Nigella seed cultivated in Egypt

Characteristic	Determined values (M±S.D.)[a]	Values reported in the literature[b]					
		1	2	3	4	5	6
Seed index[c] (g)	2.21±0.14	2.26	nd	nd	nd	nd	nd
Seed volume[d] (cm^3)	2.76±0.10	2.85	nd	nd	nd	nd	nd
Bulk denisty (g/cm^3)	0.80±0.01	0.79	nd	nd	nd	nd	nd
Moisture content (%)	7.0±0.5	6.4	5.5	5.6	nd	3.8	5.8
Crude protein[e] (%)	20.8±1.1	20.6	21.3	nd	31.2	21.6	26.6
Ether extractable (%)	34.8±1.9	37.4	35.5	22.0	53.4	40.6	34.9
Ash content (%)	3.7±0.7	4.8	3.8	nd	nd	4.5	4.7
Total carbohydrates[f] (%)	33.7±0.5	30.8	34.0	nd	nd	24.9	26.9

[a] M±S.D. = mean of triplicate samples±standard deviation.
[b] (1) Babayan et al. (1978). (2) Dandik and Aksoy (1992). (3) Abdel-Aal and Attia (1993). (4) El-Dhaw and Abdel-Munaem (1996). (5) Takrruri and Dameh (1998). (6) Salem (2001).
[c] Seed index = weight of 1000 seeds in grammes.
[d] Seed volume = volume of 1000 seeds in cm^3.
[e] Crude protein = N% × 6.25.
[f] Total carbohydrates were calculated by difference.

Proximate analysis of nigella seeds showed that crude protein (20.8%), moisture content (7.0%) and total carbohydrates (33.7%) were slightly higher than those previously reported in the literature, while, ash content (3.7%) and extractable lipid (34.8%) of the seeds were lower than those previously reported. These differences may be related to the variations of cultivated regions.

3.2. Nigella seed oil

Crude oil extracted from nigella seed by cold press was lower than that gained by solvent extraction (Table 2). This may be attributed to the greater ability of the organic solvent (compared to cold pressing) to extract most of the lipid-components, including oleoresins that are present in nigella seed. These results are in full agreement with those reported by Salem (2001). As mentioned by Eskin (1988) and Salunkhe, Chavan, Adsul, and Kadam (1992), direct pressing extraction of rapeseed oil leaves a residual cake with 4–12% oil content, decreasing to 1–2% oil in the case of solvent extraction. Nevertheless, these results conflict with those reported by Üstun et al. (1990), who found no differences between the amounts and characteristics of crude oils extracted from nigella seed by two different methods (cold pressed and solvent extraction).

3.3. Physical and chemical properties of crude Nigella seed oil

Melting point (MP) of crude oil obtained by cold press (−1.7 °C) was higher than that of solvent extraction

Table 2
Physical and chemical properties of Nigella seed oil as affected by extraction method

Properties	Determined values						Values reported in the literature[a]				
	Cold Pre.			Solvent Extr.			1	2	3	4	5
Oil extraction[b]	24.76%			34.78%							
SG (g/cm^3)	0.9110±0.0003			0.9210±0.0002			0.9207	0.9201	(0.9044–0.9221)	nd	(0.9229–0.9153)
RI (at 20 °C)	1.4732±0.0001			1.4721±0.0002			1.4718	1.4728	(1.4690–1.4731)	(1.4714–1.4724)	(1.4723–1.4735)
MP (°C)	−1.7±0.6			−3.3±0.6			nd	nd	nd	nd	(−4–−5)
Colour	R	Y	B	R	Y	B					
	08	42	14	11	42	81	nd	nd	nd	nd	
FFA (as Oleic%)	11.0±0.0			6.7±0.4			15.2	0.5	(19.9–21.4)	(6.3–8.1)	(7.2–11.6)
PV ($meqO_2/kg$)	13.5±0.2			10.7±0.4			nd	nd	nd	(14.8–18.1)	(7.6–10.6)
IV (Wijs)	115±31			128±21			114.5	125	(112–122)	(107–110)	(128–129)
SN	192±2.0			203±3.0			196	189	(195–230)	(195–210)	nd
USM (%)	1.0±0.4			1.8±0.3			0.7	nd	(3.5–5.4)	nd	nd

M±S.D. = mean±standard deviation. ($n = 7$). nd = not determined
[a] (1) Gad et al. (1963). (2) Babayan et al. (1978). (3) Üstun et al. (1990). (4) Abdel-Aal and Attia (1993). (5) Salem (2001).
[b] Oil extraction = weight of extracted oil × 100/weight of seeds R = red colour, Y = yellow colour and B = blue colour.

(−3.3 °C). Also, colour values were different, with oil obtained by cold press having a golden yellow colour while oil extracted by solvent had a brownish yellow color (Table 2). This may be related to the ability of organic solvent to extract most lipid-soluble pigments and oleoresins present in nigella seed.

The estimated SG, RI, IV and SN were (0.9110, 1.4732, 115 and 192) and (0.9210, 1.4721, 128 and 203) for cold press and solvent-extracted crude oils, respectively. These values were similar to those reported previously (Table 2). On the other hand, crude oil, achieved by cold press, normally, had higher values than oil extracted by petroleum ether. Such results were reported by Salem (2001).

Both FFA content and PV of oil are valuable measures of oil quality. The FFA content of cold press and solvent extracted crude oils were 11.0% and 6.7% (as oleic acid), respectively. These results are lower than those determined by Gad et al. (1963) and Üstun et al. (1990). The high acidity of oil may be related to the nature of nigella seed, whereas many oil-bearing seeds, such as olive, palm and rice bran, contain high acidity oils (Patterson, 1989). The PV of cold press (13.5 meq/kg) and solvent extracted (10.7 meq/kg) nigella oils were found to be lower than those reported by Salem (2001) and higher than those reported by Abdel-Aal and Attia (1993).

The USM% of vegetable oils is considered to be natural antioxidants, that are able to minimize oil oxidation during handling and storage. The estimated USM% of both cold press (1.0%) and solvent-extracted (1.8%) oils were higher than those reported by Gad et al. (1963) and much lower than those reported by Üstun et al. (1990). These variations could be related to the diversity in maturity of seeds and the agricultural conditions of the cultivated area. Added to that the effect of extraction technique, because oil extracted by solvent was richer in USM than the cold press one. This may be correlated with potentiality of solvent to extract most of the lipid-associated substances, including phospholipids, sterols, fat-soluble vitamins, hydrocarbons and pigments (Bastic et al., 1978; Durkee, 1971; Salunkhe et al., 1992).

Crude oils extracted by the two different methods had similar lipid classes, but the percentage of their constituents were different (Table 3). For instance, oil extracted by organic solvent had higher FS, TG and ES fractions (5.0%, 63.2%, 4.4%) than those detected in oil achieved by cold press (3.0%, 57.5%, 2.5%). Meanwhile, the latter was markedly higher in FFA (14.2%). Nevertheless, TG represented the main lipid fraction in both types of crude nigella seed oil. The high percentage of FFA in cold press oil may be attributed to the hydrolysis action catalysed by native lipase in ground seeds (Dandik & Aksoy, 1992). This activity could be lost due to the contact of oil with organic solvent during extraction.

3.4. Fatty acid composition

The major fatty acids in nigella seed oil were myristic ($C_{14:0}$), palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) as saturated fatty acids, however oleic ($C_{18:1}$) and linoleic ($C_{18:2\ n\text{-}6}$)

Table 3
Lipid classes of Nigella seed oil as affected by extraction method

Lipid class	Cold press extraction (M±S.D.)[a]	Solvent extraction (M±S.D.)[a]
Polar lipids (PL)	3.7±0.2	4.8±0.8
Monoacylglycerol (MG)	4.8±0.7	5.7±0.5
Diacylglycerol (DG)	5.1±0.7	4.1±0.8
Free sterols (FS)	3.0±0.8	5.0±0.6*
Unknown (Un)	5.4±0.5	4.5±0.3
Free fatty acids (FFA)	14.2±0.5*	8.3±0.2
Triacylglycerol (TG)	57.5±2.0	63.2±2.2*
Sterol esters (SE)[b]	2.5±0.4	4.4±0.4*

*Significant at 0.05 level by Student's *t*-test.
[a] M±S.D. = means of 5 samples±standard deviation.
[b] SE = Sterol esters, hydrocarbons and pigments.

Table 4
Fatty acid composition of Nigella seed oil as affected by extraction methods[a]

Fatty acid	Cold-pressed	Solvent-extraction
C14:0	11.1±1.1	9.8±2.1
C14:1	tr[b]	tr[b]
C16:0	12.1±3.4	9.9±3.3
C16:1	0.5±0.1	0.7±0.5
C18:0	3.7±1.7	3.3±1.2
C18:1	18.9±5.4	20.1±6.1
C18:2	47.5±6.5	49.0±5.7
C18:3	2.1±0.4	2.7±1.1
C20:0	1.2±0.8	0.7±0.4
C22:0	0.9±0.4	0.8±0.2
C22:1	0.7±0.4	1.0±0.1
C24:0	0.2±0.1	0.3±0.1
TKFA[c]	98.9	98.3
TUFA[d]	1.1	1.7
TSFA[e]	29.2	24.8
TUFA[f]	69.7	73.5
TU/TS ratio	2.4	3.0
n-6/n-3 ratio[g]	22.6	18.0
PI[h]	52.2	55.0

[a] Fatty acids (%).
[b] Trace amounts (less than 0.2%).
[c] Total known fatty acids.
[d] Total unknown fatty acids.
[e] Total saturated fatty acid.
[f] Total unsaturated fatty acids.
[g] ω-6/ω-3 fatty acids ratio.
[h] Peroxidizability index (PI) = (% monoenoic × 0.025) + (% dienoic × 1) + (%trienoic × 2) + (% Tetraenoic × 4) + (% pentaenoic × 6) + (hexaenoic × 8).

Table 5
Fatty acid composition of crude Nigella seed oil fractions

Fatty acid	FFA	MG	DG	TG	SE
$C_{14:0}$	9.4	37.8	17.4	5.3	48.7
$C_{16:0}$	11.7	12.6	15.1	17.5	11.5
$C_{16:1}$	tr	tr	0.9	1.7	tr
$C_{18:0}$	8.3	3.3	3.9	6.5	4.8
$C_{18:1}$	28.4	10.3	14.1	15.1	7.2
$C_{18:2}$	30.7	32.0	37.2	44.0	17.1
$C_{18:3}$	0.7	0.5	0.7	1.9	1.1
$C_{20:0}$	3.5	tr	0.9	1.1	4.3
$C_{22:0}$	tr	tr	1.3	0.9	tr
$C_{22:1}$	6.6	tr	4.9	2.8	2.1
UFA	0.7	3.5	3.6	3.2	3.2

FFA, free fatty acids; MG, monoglyceride; DG, diglyceride; TG, triglyceride; FS, free sterols; UFA = unknowns.

and linolenic ($C_{18:3\ n-3}$) were the main unsaturated fatty acids (Table, 4). Likewise, measurable amounts of arachidic ($C_{20:0}$), behenic ($C_{22:\ 0}$) and lignoceric ($C_{24:0}$), as saturated fatty acids as well as palmitoleic ($C_{16:1}$) and erucic ($C_{22:1}$) as mono-unsatutated fatty acids were detected. These results are in agreement with previously published data (Abdel-Aal & Attia, 1993; Babayan et al., 1978; Gad et el, 1963; Üstun et al., 1990).

The total saturated and unsaturated fatty acids in cold press oil were 29.2 and 69.7%, respectively. The corresponding values in solvent-extracted oil were 24.8 and 73.5%. Accordingly, TU/TS ratios were 2.4 and 3.0 for cold press and solvent extracted oils, respectively. The calculated peroxidizability index (PI) according the equation of Song, Fujimoto, and Miyazawa (2000), for both crude nigella seed oils, were 52.2 and 55.0. As a result, it could be predicted that crude oil extracted by cold press is slightly more stable to auto-oxidation rancidity than crude oil extracted by solvent.

GLC analysis showed that fatty acid constituents of the main lipid classes separated onto TLC were dissimilar (Table 5). However, linoleic was the predominant fatty acid in TG (44.0%), DG (37%) and free fatty acid (30.7%) fractions, while myristic was the main fatty acid found in MG (37.8%) and SE (48.7%).

3.5. Sterols content of crude Nigella seeds oil

TLC and GLC analysis established that crude oil of nigella seeds extracted by organic solvent included significantly higher amounts of sterols than the cold press one (Tables 3 and 6). These results confirmed the data of Table 2, since Nigella seed oil, extracted by petroleum ether, had significantly higher amounts of USM than oil obtained by cold press. It is interesting to note that Nigella seed oil was rich in β-sitosterol which has been shown to inhibit the absorption of dietary cholesterol (Moghadasian & Frohlich, 1999). The ratio of β-sitosterol/campsterol could be used as index to identify the purity and authenticity of oil (El-Hinnawy et al., 1983). In the present investigation, the β-sitosterol/campesterol ratio in Nigella seed oil ranged from 22.7 to 16.3.

Table 6
Sterol fractions in crude Nigella seed oil as affected by extraction method (mg/100 g oil)[a]

Fractions	Cold press oil	Solvent-extraction oil
Cholesterol	52±9	94±17*
Campestrol	28±6	59±12*
Stigmasterol	68±11	124±19*
β-Sitosterol	636±35	960±60*
β-Sitostanol	44±10	67±12

*Significant different by student's t-test at $P < 0.05$.
[a] Data are means±SD for triplicate analysis.

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