# Characterization of phospholipid composition of black cumin (*Nigella sativa* L.) seed oil

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Black cumin (*Nigella sativa* L.) seed oil was extracted with two different solvents, *n*-hexane (H) and a mixture of chloroform/methanol (CM) (2 : 1, by volume). Amount of total lipid (TL) was higher in the CM miscelle (39.2% of seed fresh weight) than in the H extract (37.9%). Chemical characteristics as well as fatty acid profile of the TL extracts were compared and the analysis revealed that the major fatty acid was linoleic acid C18 : 2n-6 (*ca.* 57% of total fatty acid methyl esters (FAME)) followed by oleic acid C18 : 1n-9. Palmitic acid C16 : 0 was the major saturated fatty acid and detected in appreciable level. Chromatography on a silica column with solvent of increasing polarity yielded 96.1–97.2% neutral lipids (NL) and *ca.* 3% of polar lipids. Gas liquid chromatography with flame ionization detector (GLC/FID) showed that the major fatty acid present in all lipid classes was C18 : 2n-6 followed by C18 : 1n-9 and C16 : 0 acids, respectively. Phospholipid (PL) classes were separated *via* normal-phase HPLC. Separation was achieved on a silica column by gradient elution from isooctane/2-propanol (6 : 8, by volume) to isooctane/2-propanol/water (6 : 8 : 0.6, by volume) lasting 35 min with UV detection at 205 nm. The major individual PL classes were found to be phosphatidylcholine (PC; *ca.* 46–48% of total PL) followed by phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI), respectively. Phosphatidylglycerol (PG), lysophosphatidylethanolamine (LPE) and lysophosphatidylcholine (LPC) were isolated in smaller quantities. The level of saturated fatty acids, namely palmitic C16 : 0 and stearic C18 : 0 acids, was considerably higher in PL classes than in the corresponding triacylglycerols. Characterization of PL profile from *Nigella sativa* L. seed oil as well as the development of new source of PL was the primary aim of this study.

## 1 Introduction

Nonconventional seeds are being considered because their constituents have unique chemical properties and may augment the supply of edible oils. The study of minor constituents, such as PL, in these seeds is important for their effective use [1]. Unfortunately, there are no new miracle crops out there just waiting to be discovered. Discovery of niche markets will come from an understanding of what drives customer needs and wants [2]. *Nigella sativa* L. seeds, an annual spicy herb, also known as black cumin or black caraway, is a member of the Ranonculaceae family and native to some parts of the Mediterranean region. The seeds are used for edible and medicinal purposes in many countries including India, Egypt and Syria. They are used in the preparation of a traditional sweet dish, composed of black cumin paste which is sweetened with honey or syrup and in the preparation of pastry. They are also used for sprinkling on bread, flavouring of food, especially bakery products and cheese as well as a carmanitive, stomachic and diuretic agent [3, 4]. Recently, many medical properties have been attributed to the black cumin seeds and/or its oil, including antineoplastic (antitumour), antibacterial, antifungal, antihelmenthic and treatment of asthma [5–8]. More recently, a great deal of attention has been given to the *Nigella sativa* L. seed oil and thus their consumption has increased especially in Middle East countries. There are few studies [3, 9, 10] on the seed oil characteristics and fatty acid composition of *Nigella sativa* L. Babayan *et al.* [3] found that the seeds are rich in crude fat, polyunsaturated fatty acids (particularly linoleic acid) and protein. Saleh [9] reported in his study on the seeds growing in Saudi Arabia that linoleic and oleic acids were the major unsaturated fatty acids while palmitic acid was the main saturated one.

In response to the needs of food scientists, technologists and nutritionists, researchers are compiling data on PL and their fatty acids from new seed sources. No data about the PL content and their fatty acid constitution of *Nigella sativa* L. seed oil have yet been available. Phospholipids (PL) have potential as a multifunctional additive for food, pharmaceutical and industrial applications [11]. They combine nutritional and technological properties in a single substance class. This dual and synergistic function makes them ideal candidates for use in functional food. On the one hand, they act as emulsifiers, surfactants or liposome forming substances. On the other hand, they exhibit a wide range of nutritional and even preventive, if not in therapeutic activities. They have cholesterol reducing, liver protecting effects as well as brain improving functions [12]. As a class of natural antioxidants, PL represent the most controversial group of antioxidants in that many *in vivo* assays, they have been shown to be pro-oxidant. By contrast, many lipid model studies show that PL, alone and in conjunction with other antioxidants, have the ability to stabilize lipids [13, 14]. Development of new or better sources of PL will be considered the next step in the field of functional food research. In this work we are presenting the results from a comparative investigation on the content as well as the composition of lipid classes and PL classes in *Nigella sativa* L. seed oil. Development of new sources of PL to use both seed oil and its minor components effectively was the aim of our study.

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**Abbreviations:** **CM**, chloroform/methanol (2 : 1, by volume); **FAME**, fatty acid methyl esters; **GL**, glycolipids; **H**, *n*-hexane; **LPC**, lysophosphatidylcholine; **LPE**, lysophosphatidylethanolamine; **NL**, neutral lipids; **PC**, phosphatidylcholine; **PE**, phosphatidylethanolamine; **PG**, phosphatidylglycerol; **PI**, phosphatidylinositol; **PL**, phospholipids; **PS**, phosphatidylserine; **TL**, total lipids

**Keywords:** Black cumin / Lipid classes / *Nigella sativa* L. / Phospholipid composition / Seed oil

## 2 Materials and methods

### 2.1 Materials

Mature *Nigella sativa* L. seeds were obtained from Alfred Galke GmbH (Gittelde, Germany) and stored at 4 °C until ana-

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lysis. Standards used for PL identification, phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylinositol (PI) from bovine liver, phosphatidylcholine (PC) from soybean, phosphatidylglycerol (PG), lysophosphatidylcholine (LPC) and lysophosphatidylethanolamine (LPE) from egg yolk were purchased from Sigma (St. Louis, MO, USA). The boron trifluoride-methanol complex ($BF_3$ solution 10% in methanol) which was used for derivatization of the fatty acids, was purchased from Merck (Darmstadt, Germany). Reagents and chemicals used were of the highest purity available.

### 2.2 Methods

#### 2.2.1 Extraction of the total lipid (TL)

Two solvents were tested as to their efficiency in extracting TL as well as lipid classes. TL from finely ground seed material was Soxhlet-extracted with *n*-hexane (H) and the second extraction was with chloroform/methanol (CM) (2:1, by volume). Under the condition of extraction with CM the extracted lipids require an addition of 0.2 volume of 0.75% aqueous sodium chloride solution. The whole was thoroughly mixed without shaking, the layers allowed to separate and the chloroform layer was recovered. The lipid extract was collected in a flask and subsequently treated with sodium sulphate to remove traces of water, after filteration the extract was taken to dryness on a rotary evaporator at 40 °C. The extracted lipid were weighed to determine the TL content and stored under nitrogen at 4 °C for further analyses.

#### 2.2.2 Chemical analysis of TL

Chemical analysis of TL extracts, including acid, peroxide, iodine and saponification values were performed in accordance with AOAC [15] methods. Phosphorus and nitrogen content of the crude oils was determined using the method of AOCS [16]. Total carbohyrate content was estimated from the hexose content by using anthrone-thiourea reagent [17].

#### 2.2.3 Fatty acid composition of TL

The fatty acids were converted to methyl esters (FAME) by heating in 10% $BF_3$-methanol, according to the procedure reported by Metcalfe *et al.* [18]. FAME were identified on a Shimadzu GC-14A equipped with FID and C-R4AX chromatopac integrator (Kyoto, Japan). The carrier gas (helium) had a flow rate of 20 mL/min, split 1:40. A sample of 1 µL was injected on a 30 m × 0.25 mm × 0.2 µm film thickness Supelco SP™-2380 (Bellefonte, USA) capillary column. The injector and flame ionization detector temperature was 250 °C. The initial column temperature was 100 °C programmed by 5 °C/min until 175 °C and kept for 10 min at 175 °C, then 8 °C/min until 220 °C and kept for 10 min at 220 °C. A comparison between the retention times of the samples with those of authentic standards, run on the same column under the same conditions, was made to facilitate identification.

#### 2.2.4 Column chromatography (CC) separation of lipid classes

TL in chloroform was separated into neutral lipids (NL), glycolipids (GL) and PL by passing through a glass column (20 mm diameter × 30 cm) packed with a slurry of activated silicic acid (70–230 mesh; Merck, Darmstadt, Germany) in chloroform (1:5 w/v) according to Rouser *et al.* [19]. The eluting solvents for NL, GL and PL were chloroform, acetone and methanol, respectively. Solvents were evaporated by using a rotary evaporator and the percentage of each fraction was determined gravimetrically.

#### 2.2.5 HPLC analysis of PL classes

Normal-phase HPLC analysis of PL classes was performed according to Singleton and Stikeleather [20] with a Solvent Delivery Module LC-9A for Shimadzu (Kyoto, Japan). The chromatographic system also included a Model 87.00 (Knauer, Berlin, Germany) Variable Wavelength Monitor detector. A 25 cm × 4 mm column (Knauer, Berlin, Germany) packed with 5 µm diameter LiChrosorb Si 60 was used. PL classes were separated with a gradient program of mixed solvents and detected at 205 nm. Solvent A was a mixed solvent of isooctane/isopropanol (6:8, by volume) and solvent B was a mixed of isooctane/isopropanol/water (6:8:0.6, by volume). Separation with a gradient starting at 100% solvent A to 100% solvent B in 35 min and regeneration of the column for next analysis with 100% A for 15 min. All solvents were ultrasonicated for 5 min prior to analysis to minimize dissolved gases. About 1–2 µg of total PL (methanol fractions obtained from CC) were injected and solvent flow was maintained at 0.7 mL/min at a column back-pressure of *ca.* 105–120 bar. PL standards were used to identify the components of seed oil PL classes *via* HPLC. The phosphorus content of the classes collected manually from the HPLC (stored at −20 °C until analysis) was determined by using the AOCS method [16]. The fatty acid composition of the PL fractions was determined by GLC/FID as perviously described. All extractions, CC, HPLC and GLC/FID runs were performed in triplicates and mean values were calculated.

## 3 Results and discussion

### 3.1 Content and chemical analysis of TL

Since lipids were readily soluble in the solvents (H and CM) used in our work no great differences in amounts of TL extracted from the seeds were expected among the solvents. However, CM extracted more lipid (39.2% of total seed weight) than H (37.9%). Perhaps the former solvent, which is more polar and more acidic than the later, dislodged and dissolved oil that is tightly bound to non lipid constituents, *e.g.*, lipid associated inside intracellular and protein-rich aleurone. Chemical characteristics of the black cumin seed oil were presented in Table 1. The results showed that iodine value was higher in the TL extracted with H than in the CM extract. The

**Table 1.** Characteristics and qualities of *Nigella sativa* L. seed oil

| Analysis | H extract | CM extract |
|---|---|---|
| Peroxide value | 0.22 ± 0.03 | 0.36 ± 0.01 |
| Acid value | 0.14 ± 0.02 | 0.17 ± 0.02 |
| Saponification value | 166 ± 1.56 | 223 ± 3.73 |
| Iodine value | 48.4 ± 0.32 | 46.2 ± 0.19 |
| Phosphorus (%) | 0.01 ± 0.001 | 0.04 ± 0.005 |
| Total carbohydrate (%) | 0.54 ± 0.02 | 0.73 ± 0.05 |
| Nitrogen (%) | 0.05 ± 0.002 | 0.10 ± 0.007 |

Values are given as mean ± SD for $n = 3$ samples

**Table 2.** Fatty acid composition (% of total FAME) of *Nigella sativa* L. seed oil

| Fatty acid | H extract | CM extract |
|---|---|---|
| C16:0 | 13.0 ± 0.03 | 13.1 ± 0.05 |
| C18:0 | 3.16 ± 0.01 | 3.22 ± 0.02 |
| C18:1n-9 | 24.1 ± 0.03 | 23.9 ± 0.03 |
| C18:2n-6 | 57.3 ± 0.04 | 57.0 ± 0.06 |
| C20:1n-9 | n.d.[a)] | 0.30 ± 0.002 |
| C20:2n-6 | 2.44 ± 0.01 | 2.48 ± 0.02 |

a) n.d., not detected
Values are given as mean ± SD for $n = 3$ samples

iodine value, which provides a measure of degree of oil unsaturation, has been commonly used as a means of predicting shelf-life. Lower iodine value of *Nigella sativa* L. seed oil confers to a more stable oil with a longer shelf-life. However, acid, peroxide and saponification values as well as phosphorus and carbohydrate content were higher in the CM extract. It is known that lipid extraction using a non-polar solvents such as H only yields the free lipids but only part of the polar lipids [21, 22].

### 3.2 Fatty acid composition of TL

According to the results shown in Table 2 the major components of fatty acids were linoleic (C18:2n-6) and oleic (C18:1n-9) acids. These extracts also contain appreciable amounts of saturated normal chain fatty acid, especially palmitic acid (C16:0). The dominating fatty acid was linoleic acid C18:2n-6 which accounted for *ca.* 57% of the total FAME. The second major fatty acid was oleic acid C18:1n-9 (23.9–24.1%). The ratio of linoleic acid to oleic acid was more than 2:1. This result agreed that those reported in soybean oil (C18:2n-6 = 52%, C18:1n-9 = 25%) and in corn oil (C18:2n-6 = 58.7%, C18:1n-9 = 26.6%). The content of the both saturated fatty acids palmitic (*ca.* 13%) and stearic (*ca.* 3%) was comparatively lower. On the other hand, the unsaturated fatty acids amounted to more than 83% of the total fatty acid content of the lipids extracted with H and CM. The results of this investigation agree with those reported by Babayan *et al.* [3] and Saleh [9]. However, negligible amount of gadoleic acid (C20:1n-9, *cis*-9-eicosenic acid) = 0.3% was detected in the CM extract and not reported by Babayan *et al.* [3] while myristic (C14:0) = 0.9%, myristoleic (C14:1) = 0.18%, palmitoleic (C16:1) = 0.3%, arachidic (C20:0) = 0.14%, linolenic (C18:3) = 0.3% and lignoceric (C24:0) = 1.08% were detected by Saleh [9] but absent in our study. The source of this variability may be genetic (plant cultivar, variety grown), seed quality (maturity, harvesting-caused damage and handling/storage conditions), oil processing variables, or accuracy of detection and quantitative techniques.

### 3.3 Lipid classes and their fatty acid profile

The classic procedure performed to separate lipid mixture uses solvents of increasing polarity with column of silicic acid. In this procedure, PL are eluted from a column with methanol following elution of the other lipid classes. The importance of determination of lipid classes and their fatty acid composition is reflected in the utilization of each fraction in the industry. Table 3 gives the proportions of lipid classes present in the *Nigella sativa* L. seed oil (H and CM extracts) as well as their fatty acid profile. The major lipid class found in our study was NL, which constitute 96.1–97.2% of the TL, while polar lipids constitute *ca.* 3%. As expected, the level of polar lipids was higher in CM extract than in H extract. For PL, the weight percentage in the CM extract was 3 times that of H extract. Not only did CM extract the most lipid, but it also extracted much minor constituents, especially in our study polar lipids. The fatty acids present in NL and polar lipids resemble each other in the examined samples. Fatty acid profile was characterized by excepionally high levels of linoleic acid C18:2n-6 in all lipid classes, while oleic acid C18:1n-9 fall in the lower range. Palmitic acid C16:0 was the major saturated fatty acid in all classes and the other saturated fatty acid (stearic acid, C18:0) was found in small amounts. NL resemble polar lipids in the ratio of saturated fatty acids to unsaturated fatty acids which was estimated to be *ca.* 1:3. While, polar lipid fractions were characterized by high level of saturated fatty acids, which make up *ca.* 20% of total FAME in GL and more than 25% of total FAME in PL. High levels of oleic and linoleic acids, in all lipid classes could have nutritional implication.

### 3.4 HPLC of PL classes

Widely distributed in food the PL have both pro- and antioxidant effects [13, 14]. Even though they are used as food emulsifiers worldwide and although at the same time they have a very positive image, their use in functional food is still limited. Considering the amount of clinical data, there is no doubt that PL will become standard ingredients for this rapidly expanding category of food [12]. The isolation of the PL classes of

**Table 3.** Lipid classes and their fatty acid composition (% of total FAME) of *Nigella sativa* L. seed oil

| Class of lipid | H extract | | | CM extract | | |
|---|---|---|---|---|---|---|
| | NL<br>97.2[a)] ± 0.09 | GL<br>2.18 ± 0.02 | PL<br>0.32 ± 0.002 | NL<br>96.1 ± 0.06 | GL<br>2.59 ± 0.01 | PL<br>1.05 ± 0.004 |
| C16:0 | 12.2 ± 0.02 | 17.6 ± 0.03 | 23.3 ± 0.06 | 12.4 ± 0.01 | 18.4 ± 0.02 | 23.9 ± 0.04 |
| C18:0 | 3.07 ± 0.01 | 3.58 ± 0.02 | 4.23 ± 0.01 | 3.04 ± 0.02 | 2.07 ± 0.02 | 3.38 ± 0.02 |
| C18:1n-9 | 22.8 ± 0.02 | 23.6 ± 0.04 | 24.3 ± 0.04 | 22.3 ± 0.03 | 24.7 ± 0.03 | 23.9 ± 0.03 |
| C18:2n-6 | 59.9 ± 0.05 | 52.6 ± 0.05 | 45.8 ± 0.06 | 58.6 ± 0.07 | 51.8 ± 0.04 | 46.2 ± 0.07 |
| C20:1n-9 | n.d.[b)] | n.d. | n.d. | 0.67 ± 0.002 | 0.64 ± 0.002 | 0.09 ± 0.001 |
| C20:2n-6 | 2.03 ± 0.01 | 2.62 ± 0.01 | 2.37 ± 0.02 | 2.99 ± 0.02 | 2.39 ± 0.005 | 2.53 ± 0.02 |

a) g/100 g TL
b) n.d., not detected
Values are given as mean ± SD for $n = 3$ samples

**Table 4.** Phospholipid (PL) classes and their fatty acid composition (% of total FAME) of *Nigella sativa* L. seed oil

| Class of PL | H extract | | | | | | | CM extract | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PG | PE | PI | LPE | PS | PC | LPC | PG | PE | PI | LPE | PS | PC | LPC |
| | 1.51[a)] ± 0.03 | 25.1 ± 0.05 | 9.56 ± 0.01 | 1.20 ± 0.001 | 12.3 ± 0.03 | 46.1 ± 0.04 | 4.23 ± 0.01 | 1.56 ± 0.04 | 24.3 ± 0.03 | 8.99 ± 0.01 | 1.69 ± 0.003 | 11.8 ± 0.05 | 48.5 ± 0.03 | 3.16 ± 0.03 |
| C16 : 0 | 20.6 ± 0.05 | 25.4 ± 0.01 | 29.9 ± 0.03 | 24.9 ± 0.07 | 35.6 ± 0.03 | 20.3 ± 0.01 | 20.9 ± 0.02 | 24.6 ± 0.03 | 22.8 ± 0.02 | 31.7 ± 0.04 | 23.3 ± 0.04 | 32.2 ± 0.01 | 20.6 ± 0.03 | 18.8 ± 0.03 |
| C18 : 0 | 3.96 ± 0.01 | 4.39 ± 0.01 | 3.70 ± 0.03 | 2.51 ± 0.02 | 3.00 ± 0.02 | 4.66 ± 0.01 | 4.74 ± 0.01 | 3.12 ± 0.02 | 3.51 ± 0.01 | 3.86 ± 0.01 | 2.79 ± 0.03 | 2.86 ± 0.01 | 4.32 ± 0.01 | 3.49 ± 0.01 |
| C18 : 1n-9 | 25.7 ± 0.03 | 19.3 ± 0.06 | 23.5 ± 0.04 | 22.3 ± 0.03 | 18.2 ± 0.03 | 23.3 ± 0.04 | 25.1 ± 0.05 | 24.5 ± 0.06 | 20.1 ± 0.05 | 22.4 ± 0.02 | 24.6 ± 0.01 | 21.6 ± 0.03 | 22.5 ± 0.02 | 24.8 ± 0.03 |
| C18 : 2n-6 | 46.8 ± 0.05 | 48.9 ± 0.05 | 40.7 ± 0.08 | 47.6 ± 0.06 | 41.2 ± 0.08 | 49.2 ± 0.07 | 46.5 ± 0.05 | 44.7 ± 0.06 | 49.1 ± 0.05 | 39.5 ± 0.09 | 45.7 ± 0.04 | 41.1 ± 0.08 | 49.7 ± 0.06 | 49.4 ± 0.05 |
| C20 : 1n-9 | n.d.[b)] | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 0.66 ± 0.001 | 0.92 ± 0.003 | 0.59 ± 0.002 | 0.85 ± 0.003 | 0.92 ± 0.003 | 0.46 ± 0.001 | 0.85 ± 0.002 |
| C20 : 2n-6 | 2.94 ± 0.02 | 2.01 ± 0.03 | 2.20 ± 0.01 | 2.69 ± 0.01 | 2.00 ± 0.02 | 2.54 ± 0.01 | 2.76 ± 0.03 | 2.42 ± 0.03 | 2.57 ± 0.02 | 1.95 ± 0.02 | 2.76 ± 0.01 | 1.32 ± 0.01 | 2.42 ± 0.01 | 2.66 ± 0.02 |

a) g/100 g total PL
b) n.d., not detected
Values are given as mean ± SD for $n = 3$ samples

*Nigella sativa* L. seed oil had not been carried out before. HPLC offeres several advantages over TLC but none of the published HPLC techniques for separation of complex lipids met all of our objectives. UV absorption by lipids at the 200–210 nm region is due largely to the presence of double bonds. PL do not have a specific absorbance, but can be detected by monitoring the absorbance of double bonds in their fatty acid moieties. The absorption by other functional groups, such as ester carbonyl and amino, also occurs, but it is small by comparison. Being a senstive, convenient and nondestructive method, UV detection is ideal for monitoring the separation of lipids by HPLC. PL classes from *Nigella sativa* L. seed oil (H and CM extracts) were separated into seven fractions by HPLC. The seven fractions had the same retention time when compared with authentic standards and were identified as PG, PE, PI, LPE, PS, PC and LPC, respectively. Data about the qualitative and quantitative PL classes as well as their fatty acid composition are shown in Table 4.

Phosphorimetry of PL classes in both extracts revealed that PC was the most abundant class, being more than 46% of the total PL content followed by PE, PS and PI, respectively. PC and PE make up together *ca.* 75% of total PL content. PG, LPE and LPC were found to be present in insignificant amounts. The nature of fatty acids in position 1 and 2 of the glycerol backbone can vary to an extensive degree dependent on the source of the oil [12]. Analytical data showed that in all PL classes the fatty acid profile was similar to that of total PL. Furthermore, the content of saturated fatty acids, namely palmitic C16 : 0 and stearic C18 : 0 acids, was considerably higher than in the corresponding NL. Palmitic acid, moreover, was detected in higher levels in PS and PI classes ($\geqslant$30% of total FAME). Linoleic acid C18 : 2n-6 predominated as the main unsaturated acid in all PL classes followed by oleic acid C18:1n-9, whereas, eicosadienoic C20 : 2n-6 and gadoleic C20 : 1n-9 acids were presented in small quantities or in traces. Recently, Boyd [13] mentioned that the antioxidant activity of PL was associated with amine-containing PL, such as PC and PE. The ability to stabilize lipids was also affected by the chain length and degree of unsaturated of the fatty acids on the PL. Those PL with longer chain length and PL containing more saturated fatty acid were the most effective antioxidants.

## 4 Concluding remarks

*Nigella sativa* L. seed is a promising oil crop because of the specific properties of its seed oil. This study describes the components of PL in *Nigella sativa* L. seed oil and the distribution of their fatty acids. The results are important as an indication of the potentially economical utility of *Nigella sativa* L. seed oil as a new source of PL. We have shown that solvent and/or mixtures used in lipid extraction process play an important role in the amount and composition of recovered lipids. *Nigella sativa* L. seed oil, a linoleic-oleic type oil, is a good source of fatty acids for human nutrition. PL fraction have a high PC and PE concentration making this oil a good source of these components. High content of linoleic acid in all PL classes, on the other hand, make the *Nigella sativa* L. of interest in the cosmetics industry due to the effect of linoleic acid on the skin's moisture balance. It is anticipated that commercial exploitation of *Nigella sativa* L. seed oil will soon be realized.

## 5 References


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