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Physicochemical Characteristics of Nigella Seed (*Nigella sativa* L.) Oil as Affected by Different Extraction Methods

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Abstract The physicochemical properties of crude Nigella seed (*Nigella sativa* L.) oil which was extracted using Soxhlet, Modified Bligh–Dyer and Hexane extraction methods were determined. The effect of different extraction methods which includes different parameters, such as temperature, time and solvent on the extraction yield and the physicochemical properties were investigated. The experimental results showed that temperature, different solvents and extraction time had the most significant effect on the yield of the Nigella oil extracts. The fatty acid (FA) compositions of Nigella seed oil were further analyzed by gas chromatography to compare the extraction methods. The C16:0, C18:1 and C18:2 have been identified to be the dominant fatty acids in the Nigella seed oils. However, the main triacylglycerol (TAG) was LLL followed by OLL and PLL. The FA and TAG content showed that the composition of the Nigella seed oil extracted by different methods was mostly similar, whereas relative concentration of the identified compounds were apparently different according to the extraction methods. The melting and crystallization temperatures of the oil extracted by Soxhlet were −2.54 and −55.76 °C, respectively. The general characteristics of the Nigella seed oil obtained by different extraction methods were further compared. Where the Soxhlet extraction method was considered to be the optimum process for extracting Nigella seed oil with a higher quality with respect to the other two processes.

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Introduction

Nigella (*Nigella sativa* L.) also known as black cumin is an annual herbaceous plant belonging to the Ranunculaceae family. The plant is indigenous to Mediterranean areas, through it is grown in other parts of the world as well. The seeds of the Nigella plant are black in color and look like sesame seeds. Both the seeds and oil are used as a nutritional supplements [1]. Nigella seed oil has protective and curative actions [2]. Nigella seed oil is considered as one of the newer sources of edible oils that have an important role in human nutrition and health [3]. The seed oil has been reported to possess antitumor activity [4], antioxidant activity [5], anti-inflammatory activity [6], antibacterial activity [7] and a stimulatory effect on the immune system [2]. Nigella seed oil is also used to treat respiratory condition like bronchitis, asthma and emphysema [8]. In addition, it is used to support stomach and intestinal health as well as kidney and liver function [9]. Nigella is thought to have antihistamine-like properties that make it useful in treating congestion [10].

A great deal of attention has been focused on Nigella seed oil, thus its consumption has increased, especially in Middle Eastern countries. As mentioned in the literature, the oil has been usually produced by a hot solvent extraction method at 40–60 °C [5, 11–13] and even at 70 °C [14], using the Soxhlet extractor. The hot solvent method of extraction could affect the oil properties and may induce partial alteration of the majority of minor constituents that have many functional, antioxidative and pro-oxidative effects [15–17]. However, none of investigations has examined in detail the comparison of physicochemical properties of Nigella seed

oil as affected by different solvent extractions. The aim of this work was to compare the basic physicochemical characteristic of Nigella seed oil obtained by three different extraction methods.

Materials and Methods

Materials

Nigella sativa L. seeds were purchased from a herbal market in Isfahan, Iran. The samples were cleaned, washed, and air dried before being transported to the University of Putra Malaysia. To determine the proximate composition and oil extraction from the Nigella seed, the samples were separately milled in a heavy-duty grinder for 2 min, passed through 1–2 mm screens and were then preserved in hermetically sealed bags at −20 °C until analysis.

Methods

Oil Extraction and Preservation

Nigella seed oils were extracted using three different methods which were the Soxhlet, Hexane, and Modified Bligh–Dyer methods. The extraction procedures were replicated three times and the collected solvent was removed using a rotary evaporator at 40 °C. Finally the collected seed oils were transferred to dark bottles, flashed with nitrogen gas and stored in a freezer at −20 °C for subsequent physicochemical analyses. All analytical determinations were performed at least in triplicate whereby the values were expressed as means ± standard deviation.

Soxhlet Extraction Method

The Soxhlet method as described by AOAC method No. 963.15 [18] was used to extract the oil with petroleum ether (40–60 °C) for 8 h.

Hexane Extraction Method

Nigella seeds powder (50 g) was placed in a dark flask and homogenized with 250 ml of hexane. After mixing for 4 h in a shaker (Selecta, Spain) at a rate of 180 U/min, the mixture was centrifuged (Kubota 5220, Tokyo, Japan) at 1,000 *g* for 15 min at ambient temperature (20 °C). The supernatant was then filtered through Whatman No. 2 filter paper [3].

Modified Bligh–Dyer Extraction Method

The Modified Bligh–Dyer method with slight modification by Kim [19] which is as described as follows: Nigella seed powder (50 g) was homogenized in a Waring blender for 2 min with a mixture of 50 ml chloroform and 100 ml methanol. Then, 50 ml chloroform was added to this mixture and blended for 30 s, finally 50 ml of distilled water was added and blended for 30 s. After that the homogenate, was filtered through a Whatman No. 1 filter paper on a No. 3 using a Buchner funnel with a slight suction and the residue compressed to ensure maximum recovery of the filtrate. The combined filtrates were transferred into a decanter. After settling for a few minutes to complete the separation and clarification the bottom layer that contains the chloroform and lipid was mixed with small amount of anhydrous Na_2SO_4 (1.5–2.5 g) if it was not clear to remove traces of water. Upon completion of the oil extraction, chloroform was removed from the oil using a rotary evaporator (Eyela A-3S, Tokyo, Japan).

Moisture Content

The moisture content was determined using the AOAC [18] method No. 984.25.

Fat Content

Oil was extracted from 15 g of seed powder in a Soxhlet extractor for 8 h using petroleum ether (40–60 °C) as a solvent. The result was expressed as the percentage of lipids (on a wet weight basis) [18].

Protein Content

Total protein was determined by the Kjeldahl method. Protein was calculated using a nitrogen conversion factor of 6.25 [11]. Data were expressed as percentages on a wet weight basis.

Ash Content

About 0.5 g of powdered seed samples were ignited and incinerated in a muffle furnace (Amalgam, Sheffield, England) at 550 °C for about 12 h. The total ash was expressed as a percentage on a wet weight basis.

Carbohydrate Content

The carbohydrate content was estimated by the difference of the mean values, i.e. [100 − (protein + lipids + ash + moisture)].

Fatty Acid Composition

Fatty acid composition was analyzed according to Christie [20] by gas–liquid chromatography after derivatization of

fatty methyl esters (FAME). FAME were prepared by transesterification of oil (50 μg) with hexane (950 ul) and sodium methoxide (50 μl, 1 M) and carried out with a Hewlett-Packard 6890 Series gas chromatograph (H.P. G1530A, USA) equipped with a hydrogen flame ionization detector and a polar capillary column: BPX-70 (60 m L, 0.32 mm ID, 0.25 μm film; SGE International Pty. Ltd., Victoria, Australia). The column temperature was programmed from 115 to 180 °C at 8 °C min^{-1} with 10 min holding time. Then the temperature was raised to 240 °C at 8 °C min^{-1} with a final holding time of 10 min. The injector temperature was set at 220 °C and detector set at 250 °C. The injection mode was splitless and 1 μl of the sample was injected. Identification and quantification of FAME was accomplished by comparing the retention times of peaks with those of pure standards purchased from Supelco (Supelco™ 37 component FAME mix) and analyzed under the same conditions. The results were expressed as a percentage of the individual fatty acids in the lipid fraction.

Determination of the Triacylglycerol Profile

The TAG profile of the Nigella seed oil was obtained by non-aqueous reversed-phase high-performance liquid chromatography (HPLC) using a Waters liquid chromatograph, without first removing the FFA [21]. A Waters HPLC system controller (Waters 2695, USA) was equipped with an auto injector. The chromatogram was processed using Empower2 software. The TAG were separated using a commercially packed RP-18 column (250 × 4 mm) with particle size of 5 μm (Merck, Darmstadt, Germany) which was placed in an oven at 30 °C. The TAGs were eluted with acetone/acetonitrile (63.5:36.5, v/v) at 1 ml min^{-1} flow rate and detected with a refractive index detector (Waters, USA). The total run time was 1 h. The TAG peaks were identified based on the retention time of TAG standards (LLL, OLL, OOL, POL, PLL, POO and PPO), where L, O and P are linoleic, oleic, and palmitic acids, respectively (Sigma–Aldrich, Inc., St. Louis, CA, USA). Peak areas produced by the data integrator were used to quantify the components based on relative percentages. The TAG profile of a commercial sample of sunflower (*Helianthus annuus*) oil was analyzed and used as a comparison as describe by Ghazali et al. [21].

Specific Extinction

K232 and K270 extinction coefficients were calculated from the absorbances at 232 and 270 nm, respectively, with a UV spectrophotometer (Pharmacia biotech, Ultrospec2000 UV–Vis spectrophotometer, Cambridge, England) using a 1% solution of oil in cyclohexane and a path length of 1 cm.

Determination of Peroxide, Iodine, Saponification Values and Free Fatty Acid Contents

The peroxide value (PV), iodine value (IV), saponification value (SV) and free fatty acids content (FFA) of the extracted oil were determined according to the method of the AOCS [22]. Determination of the refractive index (RI) (at 25 °C) was determined with a refractometer (ATAGO hand refractometer, N-3E, Japan) according to AOAC method No. 921.08 [18].

Thermal Behavior Determination

To determine the thermal properties of the lipid, a Perkin–Elmer Diamond differential scanning calorimeter (Perkin–Elmer Corp., Shelton, CT, USA) was used. Nitrogen (99.999%) was the purge gas at a flow rate of 20 ml/min. In the first step, 5–7 mg of neutral lipid sample was weighed into a volatile aluminum pan and sealed with a cover. After sealing, the sealed pan with the sample was heated to 70 °C for 2 min, cooled to −70 °C at 10 °C min^{-1} and held for 1 min isothermally. It was consequently heated to 70 °C at 10 °C min^{-1}, held isothermally for 1 min and cooled to −70 °C at 10 °C min^{-1}. An empty, hermetically sealed aluminum pan was used as the reference. Before sample analysis, the base line was obtained with an empty, hermetically sealed aluminum pan. The melting/crystallization point was determined as the temperature at which the sample was entirely melted/solidified and was noted from the heating/cooling program.

Results and Discussion

Proximate Analysis of Nigella Seed Powder

The average proximate composition of the Nigella seed powder and some literature referencess are shown in Table 1. Proximate analysis of the Nigella seed showed that it contained 5.40% moisture, 37.33% lipids, 20.02% protein, 6.72% ash and 30.53% carbohydrate (by difference). Proximate analysis of whole mature Nigella seeds from the literature showed that the moisture content ranged from 3.8 to 7.4%, ether-extractable lipids from 22 to 53.4%, crude protein from 20.6 to 31.2%, ash from 3.7 to 4.8%, and carbohydrates from 24.9 to 34% [8, 13, 23–27]. In this case, the protein content was slightly lower than cases found in the literature, in contrast to the ash content which was higher than cases reported in the literature. These differences may be associated with variations in cultivation and climate differences within the region. The result also indicated that Nigella seed is a good source of protein and lipids for human consumption.

Nigella Seed Oil Yield

The yields of crude oil extracted using three different extraction methods are shown in Table 2. Crude oil extracted from Nigella seed using Soxhlet method had the highest yield (37.33%) followed by Modified Bligh–Dryer (33.24%) and Hexane extraction method (31.76%), respectively. This may be certified to the high ability of the petroleum ether (compared to chloroform–methanol and hexane) to extract some of lipid components in Nigella seed as well as extraction period (8 h) comparing with other extraction methods (0.5–4 h). The color of crude oil obtained from Soxhlet extraction method was brownish yellow which was more desirable compared to the crude oil extracted using Modified Bligh–Dyer and Hexane method.

Physicochemical Properties of Nigella Seed Oil

The data of Table 3 show the physicochemical characteristic of Nigella seed oils and their values in the literature. FFA and PV are used as the most important indicator for seed oil quality. The FFA value of Nigella seed oil extracted with the Soxhlet, Modified Bligh–Dyer, and Hexane methods were 6.55, 3.48 and 9.29 mg/g respectively. A low FFA value indicates that the stability of the oil extracted using the Modified Bligh–Dyer method was

Table 1 Proximate analysis of Nigella seed powder

Proximate composition	Determined value	Valued in the literature		
		Atta [8]	Dandik and Aksoy [23]	Salem [25]
Moisture (%)	5.40 ± 0.13	7.00	5.50	5.80
Ether extract lipid (%)	37.33 ± 0.15	34.80	35.50	34.90
Protein (%)	20.02 ± 0.27	20.80	21.30	26.60
Ash (%)	6.72 ± 0.02	3.70	3.80	4.70
Total carbohydrate (%)	30.53	33.70	34.00	26.90

Values are means ± SD of triplicate

Table 2 Characteristic of Nigella seed oil obtained by different extraction methods

Extraction methods	Yield (%)	Extraction periods (min)	Color	Organic solvent used
Hexane	31.76 ± 0.64[a]	240	Pale yellow oil	Hexane
Soxhlet	37.33 ± 0.15[c]	480	Brown-yellow oil	Petroleum ether
Modified Bligh–Dyer	33.24 ± 0.59[b]	30	Pale yellow oil	Chloroform/methanol

Means with different lowercase letters within a column in each extraction method are significantly different ($P < 0.05$). Values are means ± SD of triplicate

Table 3 Effects of extraction on physicochemical parameters of Nigella seed oil

Physicochemical parameters	Extraction methods			Determined valued range in the literatures[a]
	Hexane	Soxhlet	Modified Bligh–Dyer	
FFA (as oleic %)	9.29 ± 0.02[a]	6.55 ± 0.01[b]	3.48 ± 0.01[c]	0.5–21.4
PV (meq/kg)	9.78 ± 0.04[a]	6.72 ± 0.04[b]	5.60 ± 0.05[c]	7.6–18.1
IV (g I_2/100 g oil)	120.35 ± 0.05[c]	124.77 ± 0.15[a]	122.00 ± 0.18[b]	107–129
SV	191.67 ± 2.60[a]	188.58 ± 0.99[b]	184.63 ± 3.91[c]	189–230
K 232	0.72 ± 0.02[a]	0.70 ± 0.016[b]	0.60 ± 0.03[c]	ND
K 270	0.30 ± 0.00[a]	0.20 ± 0.00[b]	0.17 ± 0.01[c]	ND
RI at 25 °C	1.467 ± 0.00[a]	1.468 ± 0.00[a]	1.491 ± 0.00[b]	1.469–1.473
Viscosity (mPa s)	6.08 ± 0.25[a]	5.50 ± 0.36[a]	7.95 ± 0.20[b]	ND

Means with different lowercase letters within a row in each extraction method are significantly different ($P < 0.05$). Values are means ± SD of triplicate

ND not determined, *RI* refractive index

[a] Ramadan [1], Atta [8], Abdel-Aal and Attia [24], Salem [25], Babayan et al. [26], Üstun et al. [29]

higher than those other oils. In addition, these results are lower than those reported by Gad et al.[28], Üstun et al. [29] and Atta [8]. The lowest PV was also found in the oil extracted by Modified Bligh–Dyer extraction method (5.60 meq/kg) followed by Soxhlet one (6.72 meq/kg). The PV values of all the extracted samples in this study were not different than those reported by Salem [25] and lower than those determined by Ramadan [1], Atta [8], Abdel-Aal and Attia [24]. The SV of the seed oils extracted with different solvents ranged from 184.63 to 191.67. These values were similar to those reported by Babayan et al. [26] and exhibited lower values than those results which were reported by Atta [8], Abdel-Aal and Attia [24] and Üstun et al. [29].

The IV of the Hexane extraction method was slightly low (120.35 g I_2/100 g oil) compared to the other extraction methods. All the respective values were similar to those reported previously [8, 24–26, 29]. The primary and secondary oxidation products of seed oil extracted by hexane, were higher than those of the other two extraction

Table 4 Effects of extraction on fatty acid composition (% of total fatty acids) of samples of Nigella seed oil

Fatty acid composition	Extraction		
	Hexane	Soxhlet	Modified Bligh–Dyer
C 10:0	1.13 ± 0.00[b]	1.12 ± 0.00[b]	0.91 ± 00[a]
C 14:0	1.30 ± 0.00[b]	1.02 ± 0.00[a]	1.36 ± 0.00[c]
C 14:1	ND	ND	ND
C 16:0	15.00 ± 0.00[c]	14.11 ± 0.00[a]	14.70 ± .03[b]
C 16:1	ND	ND	ND
C 18:0	3.04 ± 0.00[b]	3.00 ± 0.00[a]	3.40 ± 0.00[c]
C 18:1n-9	21.73 ± 0.00[b]	21.55 ± 0.00[a]	21.94 ± 0.09[c]
C 18:2n-6	55.65 ± 0.01[a]	56.71 ± 0.03[b]	55.40 ± 0.14[a]
C 18:3n-3	2.24 ± 0.14[a]	2.52 ± 0.03[a]	2.45 ± 0.00[a]
∑ SFA	20.50	19.25	20.40
∑ MUFA	21.73	21.55	21.94
∑ PUFA	57.90	59.23	57.85
TU	79.63	80.78	79.79
TU/TS	3.88	4.20	3.91
n-6/n-3	24.84	22.50	22.61

Means with different lowercase letters within a row in each extraction method are significantly different ($P < 0.05$). Values are means ± SD of triplicate

ND not detected

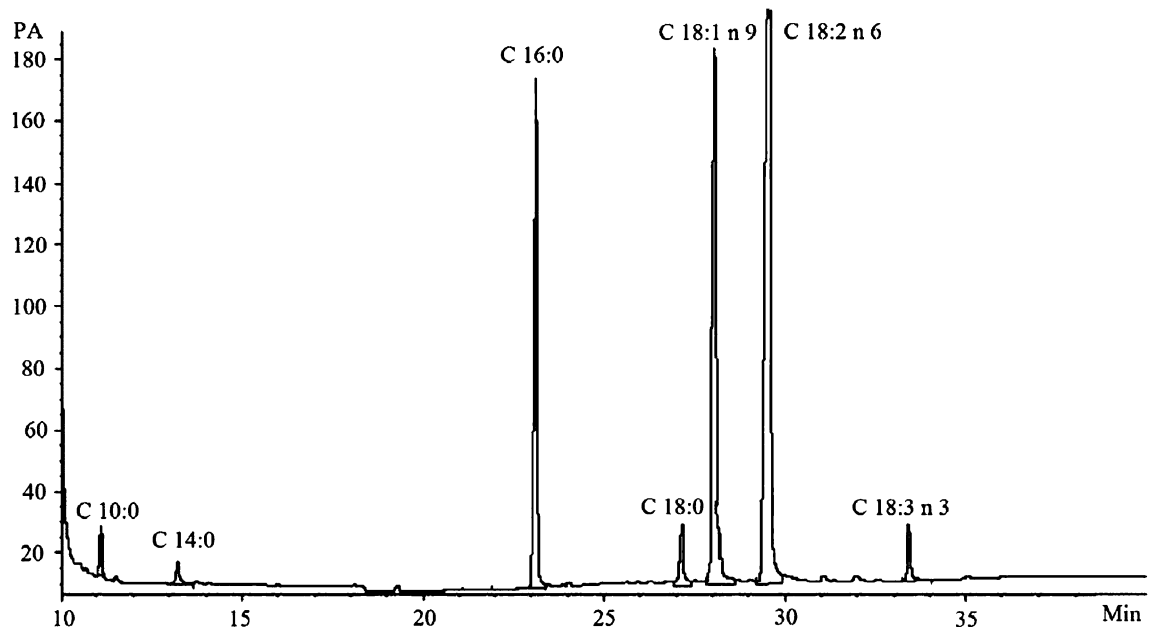

Fig. 1 GC profile of fatty acid composition in Nigella seed oil (Soxhlet)

methods. With respect to the absorbance at 232 and 272 nm, the Nigella seed oil seemed to show lower oxidation [30]. Whereas, the refractive index (RI) of the extracted oil from Nigella seed was similar to that reported by Cheikh Rouhou et al. [31]. The viscosity of Nigella seed oil extracted by the Modified Bligh–Dyer had the highest value (7.95 mPa s) in comparison with the other studied extraction methods.

Fatty Acid Profile of Nigella Seed Oils

The fatty acids profile of Nigella seed oils is shown in Table 4. The most abundant fatty acids found in the Nigella seed oil were palmitic (C16:0) as saturated fatty acid and oleic acid (C18:1) and linoleic acid (C18:2) as unsaturated fatty acids (Fig. 1). These finding are generally in agreement with those reported by Ramadan and Morsel [32] Atta [8] and Abdel-Aal and Atia [24]. The highest ratio of unsaturated to saturated (U/S) fatty acids was detected in oil extracted using the Soxhlet method (4.20%) in comparison to the other studied extraction methods. The U/S ratio of the oil in the current study showed unsaturated fatty acids in all extracted seed oils than that reported by Atta [8], Cheikh-Rouhou et al. [31]. Table 4 shows that the n-6/n-3 ratio for Nigella seed oils ranged between 22.50 and 24.84. the value of n-6/n-3 obtained is significant enough to indicate the benefits of Nigella seed oil to human health.

Table 5 Triacylglyceride profile in Nigella seed oil

TAG	Content (%)		
	Hexane	Soxhlet	Modified Bligh–Dyer
LLL	20.60 ± 0.47[a]	19.23 ± 2.15[a]	19.90 ± 0.21[a]
OLL	16.58 ± 0.40[a]	16.97 ± 0.02[a]	16.07 ± 0.17[a]
PLL	18.51 ± 0.58[a]	12.40 ± 0.04[b]	17.51 ± 0.24[a]
OOL	9.48 ± 0.23[a]	9.38 ± 0.00[a]	9.06 ± 0.17[a]
POL	13.99 ± 0.34[b]	10.78 ± 0.01[a]	13.23 ± 0.19[b]
POO	1.35 ± 0.06[a]	ND	1.86 ± 0.05[b]
PPO	1.75 ± 0.39[a]	1.56 ± 0.06[a]	4.22 ± 0.06[b]
Others	17.74	29.68	18.15

Means with different lowercase letters within a row in each extraction method are significantly different ($P < 0.05$). Values are means ± SD of triplicate

ND not detected, *L* linoleic, *O* oleic, and *P* palmitic acid

Triacylglyceride Profile of Nigella Seed Oils

Table 5 shows the distribution of TAGs determined by HPLC, while Fig. 2 shows a typical TAG profile of Nigella seed oil. The data in the same Table show TAG of Nigella seed oils contained LLL in the range of 19.90–20.60% as the most prominent TAG, followed by OLL (16.07–16.97%) and PLL (12.40–18.51%). They also contained small quantities of POO and PPO. But the oil extracted by the Soxhlet method did not show POO. Unknown TAG constituted from 17.74 to 29.68% of the total TAG content in three different extraction methods. It can be seen that there are lots of similarities among the three oils in terms of the TAG profile that are present.

Thermal Behavior of Nigella Seed Oils

The two physical events which are used to characterize the thermal behavior of lipid samples are melting and crystallization, require the intake or release of the thermal enthalpy. DSC is very suitable for verifying these physical properties of the lipid samples. The melting and cooling points of Nigella seed oil extracted by the Soxhlet method were −2.54 and −55.76 °C, respectively (Figs. 3, 4). The values of the melting and cooling points of lipids extracted by the Hexane and Modified Bligh–Dyer methods were −1.14, −51.97 and −2.41, −54.96 °C, respectively. Based on the analysis of DSC, the results revealed

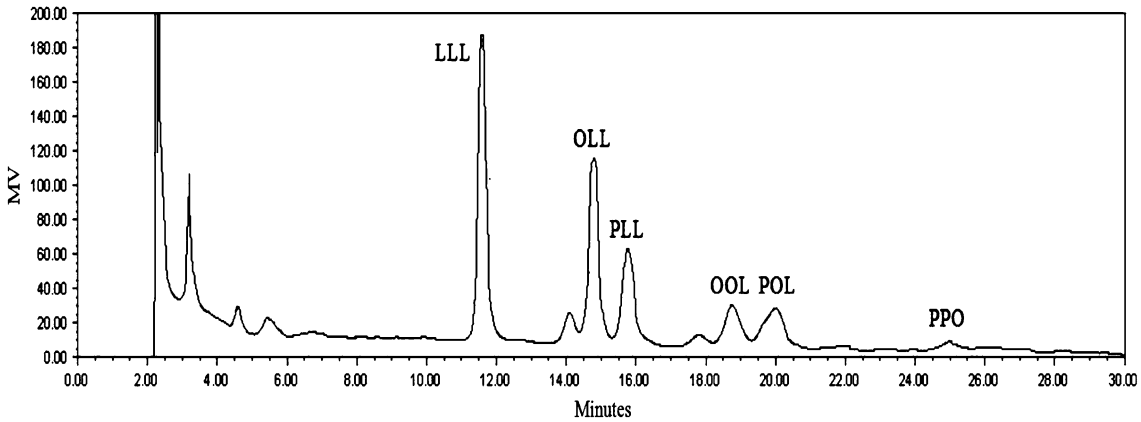

Fig. 2 HPLC Chromatogram of triacylglycerol of Nigella seed oil using Soxhlet method

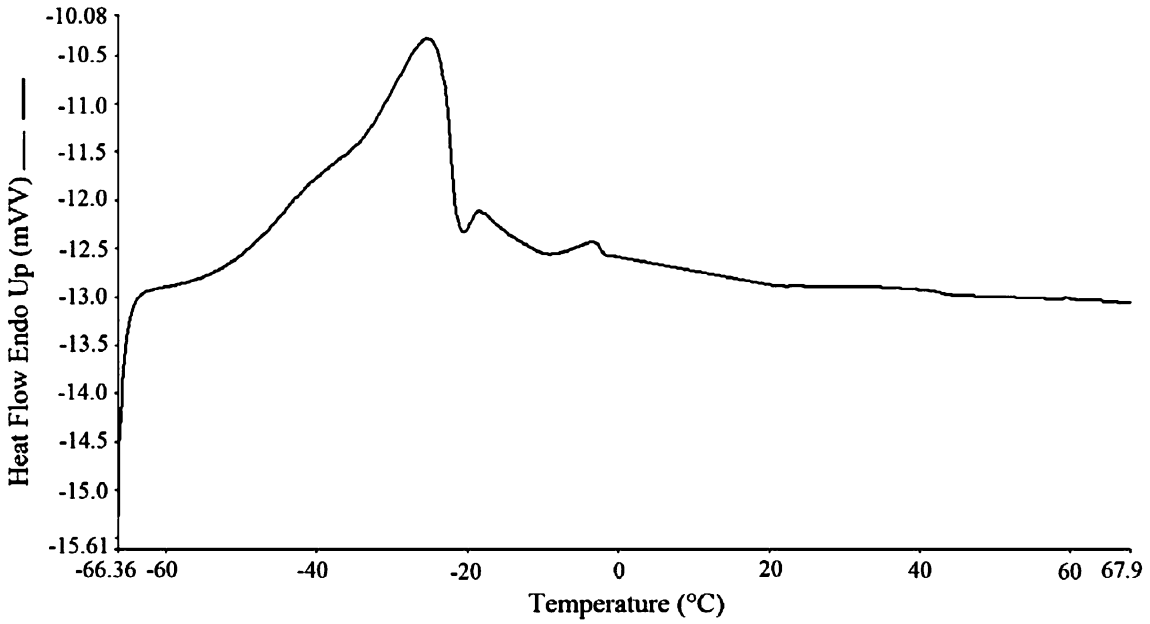

Fig. 3 Melting profile of Nigella seed oil obtained using the Soxhlet extraction method

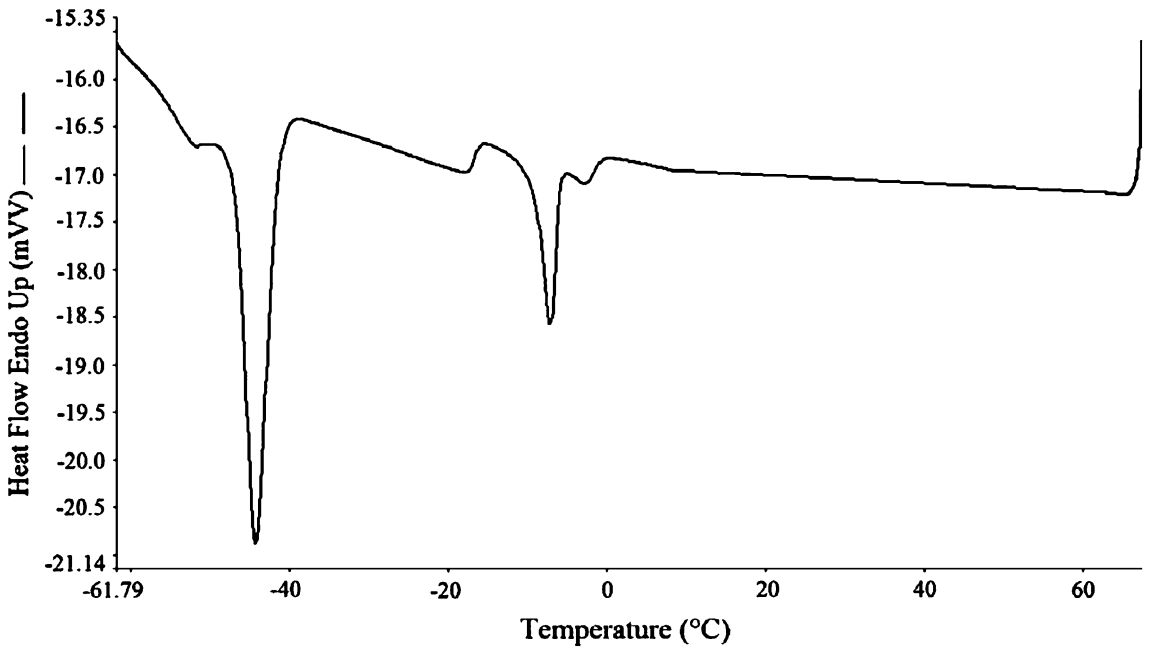

Fig. 4 Cooling profile of Nigella seed oil obtained using the Soxhlet extraction method

that most of the extracted Nigella seed oils have a high degree of unsaturated fatty acids with a high percentage of linoleic acid.

Conclusions

This study revealed that the conventional Soxhlet extraction method is the best method for extracting oil from Nigella seed compared to the other two extraction methods studied (Modified Bligh–Dyer method and Hexane extraction). The extracted oil was influenced by time, temperature and type of solvent. The Soxhlet extraction method offers many advantages, for example, it increases the yield by 4.09–5.57% compared with the other methods. The extracted oil has a higher percentage of unsaturated fatty acid and a higher n-6/n-3 ratio. Consequently, the Soxhlet extraction method can be considered as the

optimum process for Nigella seed oil extraction since the method enhances the yield and quality.

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