

## Potentials of enhancing the physicochemical and functional characteristics of *Nigella sativa* oil by using the screw pressing technique for extraction

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**SUMMARY:** In the current investigation the crude oil of *Nigella sativa* was extracted from seeds using hydraulic and screw pressing techniques. Different parameters were evaluated in order to find out the appropriate technique to enhance the physicochemical and functional-related characteristics of the extracted crude oil. Results showed that the acid and peroxide values were significantly lower in the screw pressed oil (SPO) than in the hydraulic pressed oil (HPO). The total phenolic content of the SPO was significantly higher than that of HPO. Evaluation of the oxidative stability using the Rancimat test showed that SPO recorded a much higher oxidative stability index (40.07 h) than HPO (0.51 h). The yield of the volatile oil fraction and its contents of thymoquinone isolated from the SPO were higher than that from the HPO. Biological evaluation revealed that the SPO had significantly higher antimicrobial activity than HPO against *Listeria monocytogenes* and *Staphylococcus aureus* at 40  $\mu$ L/well.

**KEYWORDS:** Crude oil; Functionality; Hydraulic pressing; *Nigella sativa*; Screw pressing

**RESUMEN:** Mejoras potenciales de las características físico-químicas y funcionales del aceite de *Nigella sativa* mediante extracción con prensa de tornillo. En la presente investigación se extrajo el aceite crudo de *Nigella sativa* de las semillas utilizando técnicas hidráulicas y de prensado de tornillo. Se evaluaron diferentes parámetros para conocer la técnica apropiada que potencie las características físico-químicas y funcionales del aceite extraído. Los resultados mostraron que los valores de ácido y peróxido fueron significativamente más bajos en el aceite de presión con tornillo (SPO) que en el aceite prensado hidráulico (HPO). El contenido fenólico total del SPO fue significativamente mayor que el de HPO. La evaluación de la estabilidad oxidativa mediante Rancimat demostró que el aceite SPO tiene un índice de estabilidad oxidativa mucho más alto (40,07 h) que el HPO (0,51 h). El rendimiento de la fracción volátil y su contenido de timoquinona aislada del aceite SPO fueron superiores a los del HPO. La evaluación biológica reveló que el aceite SPO tenía una actividad antimicrobiana significativamente mayor que el HPO contra *Listeria monocitogenes* y *Staphylococcus aureus* a 40  $\mu$ L/pocillo.

**PALABRAS CLAVE:** Aceite crudo; Funcionalidad; *Nigella sativa*; Prensado hidráulico; Prensado mediante tornillos

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## 1. INTRODUCTION

Plant oils are among the main sources of supplying humans with energy, fatty acids and oil soluble vitamins. Some of these oils are characterized by additional functionalities and biological activities which makes them suitable for consumption by a wide population sector as a dietary supplement for health promotion or protection against some diseases. Examples of these functional oils which already exist in the supplements' market include flax, pumpkin and *Nigella sativa* oils. The functionality of these oils (in terms of protective and/or therapeutic activities) has been previously reported (Mason *et al.*, 2015; Biljana *et al.*, 2014; Al-Okbi *et al.*, 2015), respectively.

Functional oils, like many other plant oils, are stored in the seeds; therefore they must be processed to extract their content of oils. Solvent extraction is a large scale method and the industrial method of choice for efficient extraction of seed oil with a yield reaching over 98.0% (Walkelyn and Wan, 2006). However, the use of organic solvents is considered to be a subject of consideration particularly in producing functional oils due to environmental consideration (solvent vapors) and the potentials of solvent residue in the final product. Due to these considerations, other traditional safe extraction methods are adopted for extracting functional oils from seeds such as the hydraulic and the screw pressing techniques. The hydraulic pressing technique is a traditional batch method which is considered to be suitable for small scale facilities and is based on compression forces that are generated by using mechanical pressure (Willems *et al.*, 2008). Later on, the screw pressing technique was developed to be a continuous extraction technique with more oil recovery, which makes it suitable for medium scale production levels. Screw pressing is characterized by its capacity to extract a greater proportion of oil from the same amount of seeds than the hydraulic pressing technique due to the combination of shear forces (due to friction) and compression forces which operate together on the seeds (Akinoso *et al.*, 2009).

Based on the importance of the hydraulic and screw pressing methods as environmental friendly and safe techniques to extract functional oils for the dietary supplements' market, the authors in the current investigation set out to screen for the most appropriate expression technique in order to enhance the physicochemical and functional-related characteristics of the crude oil extracted from the seeds of *N. sativa*. This oil was chosen among other functional oils due to its dietary characteristics as a rich source of essential fatty acids such as linoleic (54.0–70.0%) and oleic (15.0–24.0%), (Tulukcu, 2011). In addition, the oil has protective and therapeutic potentials against some liver diseases as evident from

previous work by our group (Al-Okbi *et al.*, 2015; Al-Okbi *et al.*, 2013). *N. sativa* oil also has a protective effect against oxidative stress (Develi *et al.*, 2014) and other pre-clinical and clinical effects (Gholamnezhad *et al.*, 2016). These functional characteristics make the oil of *N. sativa* an ideal candidate for complementary and continuous investigations aimed at obtaining the most functional benefits of the oil.

In previous reports in the literature the effect of different extraction methods on some physicochemical characteristics of *N. sativa* crude oil was evaluated (Kiralan *et al.*, 2014; Zzaman *et al.*, 2014; Khoddami *et al.*, 2011). However, to our knowledge, there are no trials found so far which examine the effect of hydraulic and screw pressing on the physicochemical characteristics of that crude oil, which will be addressed in the current study. In addition, different parameters related to the functional properties of *N. sativa* oil such as the total phenolic content, thymoquinone content in the volatile oil fraction, the inherent antioxidant capacity of the oil to protect itself against oxidation, and the antimicrobial activity will also be evaluated for oils from both extraction methods. This will lead ultimately to obtaining an overview on the most appropriate pressing technique that leads to enhancing the physicochemical and functional-related properties of *N. sativa* crude oil.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Ten kg of mature dried seeds of black cumin (*Nigella sativa*) were purchased from a specialized herbal retail store located in Cairo, Egypt. Gallic acid, Folin-Ciocalteu reagent, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), tet-butylhydroquinone (TBHQ) and a series of straight chain alkanes (C<sub>8</sub>-C<sub>26</sub>) kit were purchased from Sigma-Aldrich Chemical Co., (St. Louis, USA). All the solvents and other chemicals used were of analytical grade.

### 2.2. Extraction of *N. sativa* crude oil

#### 2.2.1. Extraction by hydraulic pressing

*N. sativa* crude oil was extracted from the corresponding seeds by using hydraulic pressing at room temperature (Ustun *et al.*, 1990). The dry seeds were milled into a fine powder, then batches of the powder, 250 g each, were wrapped in a thick heavy-duty cloth and the oil was extracted using an in-house made hydraulic laboratory scale pressing machine manufactured at the National Research Center, Cairo, Egypt and operated at its maximum pressure (3500 psi) for 1.0 h at room temperature. The clear extracted crude oil of *N. sativa* was collected

in dark brown glass bottles equipped with screw caps and stored in the refrigerator until analyzed in less than 1 week. The obtained hydraulic pressed oil is termed (HPO) in this study. The yield percentage of HPO relative to the weight of the seeds was calculated. All extractions were performed in triplicate.

#### 2.2.2. Extraction by screw pressing

*N. sativa* crude oil was also extracted from the same batch of seeds by using a commercial scale expeller screw pressing machine located at the same herbal retail store from which the seeds were purchased (Al-Okbi *et al.*, 2015). The seeds were exhaustively screw pressed for two successive cycles and the extracted crude oil of *N. sativa* was filtered and received in dark brown glass bottles equipped with screw caps and stored in the refrigerator until analyzed in less than 1 week. The obtained screw pressed oil is termed (SPO) in this study. The yield percentage of SPO relative to the weight of the seeds was calculated. All extractions were performed in triplicate.

#### 2.2.3. Extraction by hexane

*N. sativa* crude oil was also exhaustively extracted from the same batch of seeds by using hot hexane. The ground seeds were placed in extraction thimbles and extracted with n-hexane at 60 °C for 3 h using a Soxhlet apparatus. The solvent was dried over anhydrous sodium sulfate to absorb any traces of water and then evaporated at 40 °C under vacuum to obtain the crude oil. The product was weighed and the yield was then calculated based on the weight of the extracted seeds. The yield percentage of the crude oil relative to the weight of the seeds was calculated by dividing the weight of the extracted crude oil by the weight of its corresponding seeds x 100. All extractions were performed in triplicate.

### 2.3. Physicochemical characteristics of the crude oils

Acid value, peroxide value, saponification value and iodine number were determined according to the American Oil Chemists' Society (AOCS, 1997).

The colors of the HPO and SPO were determined by the Hunter colorimeter (HunterLab, Labscan XE) which was used to measure the color using CIE L\*a\*b\* coordinates (CIELab color scale) according to the American Oil Chemists' Society (AOCS, 1998). The formation of non-enzymatic browning color in the crude oils from both extraction techniques was measured spectrophotometrically using a UV/visible spectrophotometer, (4054-LKB-Biochrom Comp., London, England) at 420 nm using ethanol as a blank according to a previous method (Birk *et al.*, 1998). All evaluations were performed in triplicate.

### 2.4. Total phenolics in the crude oils

The total phenolic compounds in the extracted HPO and SPO were determined colorimetrically at 725 nm, using the Folin-Ciocalteu reagent (Gutfinger, 1981). Calculations of the total phenolic content in HPO and SPO were done using different concentrations of gallic acid ranging from 0.0 to 450.0 mg/mL. All of the samples were run in triplicate.

### 2.5. Oxidative stability of the crude oils

The oxidative stability of the HPO and SPO was conducted using a Rancimat instrument model 679 Metrohm AG, Switzerland, according to Lutterodt *et al.*, (2010). The accelerated oxidation of the different *N. sativa* crude oils was carried out at 100 °C with an air flow rate of (20 l/h), using 6 mL of each crude oil sample. The time period (induction time) required for the crude oil to develop a measurable rancidity which is manifested by a point of inflection on the curve of conductivity against time was determined and called the oxidative stability index (hour). All evaluations were performed in triplicate.

### 2.6. Antimicrobial activity of the crude oils

HPO and SPO were subjected to an antimicrobial evaluation against some food borne pathogenic bacteria. The tested microorganisms were provided from the culture collections of the Microbiological Department National Research Center (NRC) Dokki, Giza, Egypt. These include two strains of Gram-positive bacteria *Staphylococcus aureus* (ATCC 43300), *Listeria monocytogenes* (ATCC 35152) and one strain of Gram-negative bacteria *Escherichia coli* (ATCC 27325).

The antimicrobial evaluation was performed using the agar well diffusion method (Pérez *et al.*, 1990). In detail, 0.1 mL of the diluted inoculums ( $10^7$  CFU/mL) of each test organism was spread on tryptone soy agar (TSA) plates. Each of the HPO and SPO was diluted separately with an equal volume of absolute isopropanol (1,1, volume ratio) to decrease its viscosity and facilitate diffusion through the agar gel. Then, 40 µl and 80 µl of each crude oil/isopropanol mixture were transferred into 5 mm diameter wells that were previously punched into the agar medium. This volume contained 20 µL and 40 µL of each crude oil/well. The Petri dishes were incubated at 37 °C for 18 h. The antimicrobial activity was evaluated by measuring the zone of inhibition (mm) corresponding to each tested microorganism. Three replicates for each of the two concentrations were evaluated.

## 2.7. Isolation of the volatile oil fraction from the crude oils

HPO and SPO were subjected separately to a hydro-distillation process to isolate their volatile oil fractions which were co-extracted with each crude oil during the pressing process. In detail, each crude oil (100g corresponding to ~ 500g seeds) was mixed with distilled water (1:7 w/v) in a 5 l round bottom flask and distilled for three hours using the Clevenger-type apparatus. The cup that receives the pure volatile oil fraction in the side arm of the apparatus was wrapped with aluminum foil to protect the volatile oil from light. At the end of the distillation each volatile was collected, weighed and its content (yield percent) relative to the weight of its corresponding crude oil was calculated as the mean of three extraction processes  $\pm$ S.D.

## 2.8. Chemical analysis of the volatile oil fractions using GC and GC-MS

The volatile oils of *N. sativa* isolated from their corresponding HPO and SPO were first analyzed by gas chromatography (GC). Each volatile oil (20  $\mu$ L) was diluted separately in 1 mL diethyl ether in a glass vial. Then 2  $\mu$ L of this mixture were injected (at a split ratio 20:1) into a Perkin Elmer XL GC equipped with a FID. A fused silica capillary column HP1-MS (60 m  $\times$  0.32 mm  $\times$  0.25  $\mu$ m) was used to separate the different volatile oil components. The oven temperature was programmed from 50  $^{\circ}$ C to 230  $^{\circ}$ C at a rate of 2  $^{\circ}$ C/min. The injector and detector temperatures were 280  $^{\circ}$ C and 300  $^{\circ}$ C respectively. Helium was used as carrier gas at a flow rate of 1 mL/min. The volatile oil constituents were semi-quantified as percent of the total peak areas after flame ionization detection. All values were means of three injections from three different extractions  $\pm$  SD. The available authentic samples were used

to reveal the identity of the volatile oil constituents by matching their retention times with that of the eluted peaks of the volatile oils. The retention index (RI) was also calculated for each peak (Adams 2007) by using a series of straight chain alkanes (C<sub>8</sub>-C<sub>26</sub>) kit.

A gas chromatographic-mass spectroscopic analysis (GC-MS) was also conducted in order to reveal and confirm the identity of the components of the different volatile oils. The analysis took place using a Agilent Technologies 7890 A gas chromatograph equipped with a 5975 C quadrupole mass selective detector and the same column used as in the GC analysis. The conditions used for the GC-MS analysis were the same as that previously described in the GC section. The total ion current (m/z 40–400) from the mass spectrometer detector was used to identify the compounds by comparing their mass spectra with that of the in-house mass spectral electronic library.

## 2.9. Statistical analysis

The comparison between all evaluated parameters of HPO and SPO were analyzed using two independent T-tests. The content (yield %) and the chemical composition of the volatile oil fractions of HPO and SPO were expressed as the means of three measurements  $\pm$ S.D. The antimicrobial evaluation of HPO and SPO was analyzed using Mann Whitney's test ( $P < 0.05$ ). The statistical analysis was carried out using SPSS software version 22.

## 3. RESULTS

### 3.1. Effect of pressing technique on the yield and some physicochemical characteristics of the crude oils

Table 1 shows that there was no significant difference in the yield between hydraulic pressed oil

TABLE 1. Physicochemical parameters of the hydraulic and screw pressed crude oils extracted from the seeds of *N. sativa*

Parameter	Hydraulic pressed oil (HPO)	Screw pressed oil (SPO)	P value
Yield of crude oil % based on seeds	20.5 $\pm$ 0.28	21.1 $\pm$ 0.14	0.16
Acid value (mg KOH/ g oil)	64.7 $\pm$ 0.14	6.03 $\pm$ 0.02	0.001**
Peroxide value (meq. O <sub>2</sub> / kg Oil)	82.5 $\pm$ 1.27	72.0 $\pm$ 0.28	0.045**
Saponification value (mg KOH/g oil)	188.4 $\pm$ 0.71	189.7 $\pm$ 0.21	0.2
Iodine value (g I <sub>2</sub> / 100 g oil)	120.6 $\pm$ 0.28	120.7 $\pm$ 0.28	0.76
Original color Index:			
L*	3.79 $\pm$ 0.01	4.35 $\pm$ 0.01	0.002**
a*	1.67 $\pm$ 0.02	3.41 $\pm$ 0.01	0.002**
b*	3.25 $\pm$ 0.01	6.39 $\pm$ 0.01	0.001**
Color from Non-enzymatic browning:			
Absorption at 420 nm	0.0	0.27	
Total phenols*	38.0 $\pm$ 0.42	67.87 $\pm$ 0.23	0.001**
Oxidative stability index (h)	0.51 $\pm$ 0.01**	40.07 $\pm$ 0.02	0.001**

Results are expressed as mean  $\pm$  SD.

\* mg gallic acid equivalent/kg oil.

\*\* p < 0.05, means within the same row are significantly different.

(HPO, 20.5%) and screw pressed oil (SPO, 21.1%). On the other hand, determination of the crude oil yield after exhaustive seed extraction via the Soxhlet apparatus using hexane as a solvent gave an oil yield of 33.1%. This result means that hydraulic and screw pressing techniques extracted only 62.0% and 63.4% of the actual amount of the crude oil present in the seeds, respectively. Previous studies showed that the yield of SPO of *N. sativa* was 22.5% (Al-Okbi *et al.*, 2013) and that of an optimized HPO from different seeds of *N. sativa* was 22.0% (Hadjadj *et al.*, 2014) which are close to our findings.

The Data in Table 1 indicates also that the acid value (AV) of HPO was significantly higher (64.7 mg KOH/ g oil) than that of the SPO (6.03 mg KOH/g oil). The AV of SPO was just slightly higher than the standard of Codex Alimentarius Commission (1999) for edible oils which was pre-set at 4.0% (determined as oleic acid).

The peroxide value (PV) of the HPO was also significantly higher (82.5 meq. O<sub>2</sub>/kg oil) than the SPO (72.0 meq. O<sub>2</sub>/kg oil). There were no observed significant differences in the saponification and iodine values between the HPO and SPO. These two parameters are the measuring criteria for the molecular weight and the number of double bonds in the triglyceride molecule, respectively.

Regarding the effect of the pressing technique on the color of the crude oil, Table 1 show that there were significant differences in the values of *L*\* (for lightness), *a*\* (for red/green coordinate) and *b*\* (for yellow/blue coordinate) between the HPO and SPO. The oil obtained with the hydraulic pressing (HPO) showed lower values indicating lighter colors than screw pressed oil (SPO), which appears darker. In addition to the natural oil color due to the presence of pigments, there was an increase in optical density of the SPO when measured at 420 nm ( $A_{420\text{ nm}} = 0.27$ ) compared to the HPO ( $A_{420\text{ nm}} = 0.0$ ), as shown in Table 1. This indicates the formation of additional color due to the heat induced non-enzymatic browning (Millard reactions) which is initiated by the relatively high extraction temperature of the screw expression process as will be shown later.

Table 1 also indicates that the total phenolic contents of the HPO are significantly lower (38.0 mg gallic acid eq./kg oil) than the SPO (67.87 mg gallic acid eq./kg oil). In addition, the HPO showed a significantly lower oxidative stability index (0.51 h) than SPO (40.07 h), in the accelerated oxidation test (Rancimate). The phenolic content and stability toward oxidation are among the parameters which relate to the inherent antioxidant capacity of the crude oil of *N. sativa*.

It is worth noting that the temperature of the exhausted seeds after the completion of the hydraulic pressing process which took place in an air conditioned room was 25 °C ± 1. On the other hand, the temperature of the exhausted seeds (flakes) and

the oil that came from the machine during the screw pressing process was 50 °C ± 1 taking in consideration that this process took place in a non-air conditioned and open door herbal retail store in January, where the temperature of the surroundings was ~17 °C. This variation in the extraction temperatures was found to have a significant influence on the physicochemical, chemical and microbiological characteristics of both HPO and SPO as seen in this section and also in the next discussion section.

### 3.2. Effect of pressing technique on the content and chemical composition of the volatile oil fractions

Table 1 shows that the amount of the volatile oil fraction isolated from the SPO was significantly higher (2.6 ± 0.03 wt %, based on crude oil) than the one isolated from the HPO (1.78 ± 0.04 wt %, based on crude oil). The chemical composition of both volatile oil fractions isolated from HPO and SPO is illustrated in Table 2. From the table it is clear that thymoquinone (TQ) and p-cymene are the most abundant components in both volatile oil fractions. The percentage of TQ in the volatile oil isolated from the SPO was higher (68.13%) than from the HPO (56.29%). This finding adds to the advantages of SPO due to the well-known functional properties of TQ, as will be shown in the discussion section.

### 3.3. Effect of extraction technique on the antimicrobial activity of the crude oils

Table 3 shows that the SPO at its low dose (20 µL/well) and high dose (40 µL/well) inhibited the growth of *E. coli*, while the HPO was totally ineffective against this microorganism at both doses. Meanwhile, SPO showed significantly higher antimicrobial activity than HPO against *Listeria monocytogenes* at both doses. In case of *Staphylococcus aureus* there was no significant difference in the antimicrobial activity of both oils at the low dose (20 µL/well), while at the higher dose (40 µL/well) SPO showed significantly higher antimicrobial activity than HPO. From Table 3 it is also evident that both HPO and SPO are highly effective (to different degrees) against *Staphylococcus aureus* compared with *E. coli* and *Listeria monocytogenes* at all doses as evident from the higher inhibition zones shown for *S. aureus*.

## 4. DISCUSSION

In the current investigation the crude oil of *N. sativa* seeds was extracted using hydraulic and screw pressing techniques which are the traditional methods to obtain solvent-free functional oils. Theoretically, screw pressing should give a higher crude oil yield than hydraulic pressing due to the

TABLE 2. Chemical composition of the volatile oil fractions isolated from hydraulic and screw pressed crude oils of *N. sativa*

Chemical composition	IR	Relative area %	
		Volatile oil isolated from hydraulic pressed oil (HPO)	Volatile oil isolated from screw pressed oil (SPO)
$\alpha$ -thujene	924	3.66 $\pm$ 0.17	0.73 $\pm$ 0.04
$\alpha$ -pinene	930	0.79 $\pm$ 0.01	0.14 $\pm$ 0.02
sabinene	965	0.62 $\pm$ 0.01	0.21 $\pm$ 0.02
$\beta$ -pinene	969	1.35 $\pm$ 0.07	0.49 $\pm$ 0.03
$\alpha$ -terpinene	1009	0.00	0.06 $\pm$ 0.01
<b>p-cymene</b>	<b>1013</b>	<b>26.2<math>\pm</math>0.13</b>	<b>20.11<math>\pm</math>0.36</b>
limonene	1021	1.26 $\pm$ 0.02	0.79 $\pm$ 0.04
$\gamma$ -terpinene	1049	0.03 $\pm$ 0.0	0.00
cis-4-methoxythujane	1088	0.63 $\pm$ 0.0	0.53 $\pm$ 0.05
trans-4-methoxythujane	1110	3.82 $\pm$ 0.01	3.75 $\pm$ 0.20
terpinene-4-ol	1162	0.55 $\pm$ 0.0	0.72 $\pm$ 0.04
<b>thymoquinone</b>	<b>1223</b>	<b>56.29<math>\pm</math>0.21</b>	<b>68.13<math>\pm</math>0.86</b>
bornyl acetate	1269	0.11 $\pm$ 0.0	0.1 $\pm$ 0.01
Carvacrol	1278	0.9 $\pm$ 0.01	0.76 $\pm$ 0.35
$\alpha$ -Longipinene	1354	0.32 $\pm$ 0.01	0.38 $\pm$ 0.02
Longifolene	1408	1.25 $\pm$ 0.01	1.71 $\pm$ 0.02

Peak areas are means of three injections  $\pm$  SD.

TABLE 3. Antimicrobial activity of hydraulic and screw pressed crude oils extracted from the seeds of *N. sativa*

Pathogenic bacteria	Amount of <i>N. sativa</i> crude oil					
	20 $\mu$ L /well			40 $\mu$ L /well		
	HPO	SPO	Inhibition zone (mm) p value	HPO	SPO	p value
<i>E. coli</i>	0.0	11.67 $\pm$ 0.58	–	0.0	13.67 $\pm$ 0.58	–
<i>Listeria monocytogenes</i>	7 $\pm$ 0.0	11 $\pm$ 1.73	0.034*	11 $\pm$ 0.0	13.67 $\pm$ 0.58	0.034*
<i>Staphylococcus aureus</i>	28.33 $\pm$ 3.51	26 $\pm$ 1	0.38	34.33 $\pm$ 2.08	40 $\pm$ 2	0.05*
Isopropanol (control)	-ve	-ve	–	-ve	-ve	–

Results are expressed as mean  $\pm$  SD.

\*  $p < 0.05$ , means within the same row are significantly different.

combination of the shear forces beside the compression forces which work together on the seeds, as previously indicated in the introduction section. However, from Table 1 it is clear that there was no significant difference between the yield of HPO and SPO. That can be justified by the fact that the extraction efficiency of the screw pressing machine may decrease over time due to continues use, which consequently decreases the oil yield, making it comparable to that obtained by HPO.

The physical and chemical attributes of oils can directly affect their shelf life and functionality. Parameters like the acid value (AV), peroxide value (PV), oxidation stability and the color are basic criteria which are used to evaluate the quality characteristics of the oils and the suitability of the extraction method. Table 1 shows that there is a

difference in AV between HPO and SPO. That difference could be due to the de-activation (de-naturation) of lipase enzyme which is naturally present in the seeds, under the effect of the relatively high temperature ( $\sim 50$  °C) during the screw pressing extraction (see results section). As a result, the lipolysis of triglyceride under the effect of lipase during extraction could be reduced in SPO. Consequently less free fatty acids are liberated from the SPO, which justify its lower AV. On the contrary, such lipase deactivation is not likely to happen in the HPO in which the extraction temperature does not exceed that of the room temperature ( $\sim 25$  °C). This leads to greater potential for lipolytic activity during extraction which in turn can increase the AV. This justification came in accordance with a previous study (Dandik and Aksoy, 1992) although the authors estimated

the effect of temperature on lipase activity by subjecting the seeds to different temperatures in an oven instead of proceeding under real mechanical pressing conditions which generate inherent heat, as the case in our investigation.

When oils oxidize they produce a series of breakdown products at different stages starting with primary oxidation products like peroxides, which can be determined experimentally as peroxide value (PV). From the results in Table 1 it is evident that the peroxide value (PV) of the HPO is significantly higher (82.5 meq. O<sub>2</sub>/kg oil) than SPO (72.0 meq. O<sub>2</sub>/kg oil). That gave a preliminary indication that screw pressing could reduce the formation of peroxides probably by extracting some antioxidant agent(s) from the seeds of *N. sativa* to a greater extent than hydraulic pressing. Some of these agents could be the phenolic compounds which are known to possess antioxidant activity (Rice-Evans *et al.*, 1997). This postulation was supported and verified by the determination of the total phenolic content of both oils which revealed that SPO contains higher total phenolic contents (67.87 mg gallic acid/kg oil) than HPO (38.0 mg gallic acid/kg oil). That is due to the generated heat during screw pressing which led to enhanced recovery of biologically active compounds like phenolics.

Beside the contribution of total phenolic content, the increase in the PV of HPO (82.8) can also be considered as a consequence of its high AV (64.7 mg KOH/g oil, Table 1). The acid value increase is due to the increase in free fatty acids (FFA) liberated from the triglyceride by the action of lipase. These FFA are known to have a pro-oxidant action which is exerted by the carboxylic molecular group that accelerates the rate of decomposition of hydroperoxides leading to an elevation of the PV (Miyashita and Takagi, 1986). This process can also take place in the SPO but to a lesser extent than HPO due to its lower acid value (6.03 mg KOH/g oil), as shown in Table 1.

A comparison between our result from the PV evaluation and other investigations reveals the presence of a crude oil of *N. sativa* with a much lower PV value (4.3-5.6 meq. O<sub>2</sub>/kg oil), (Cheikh-Rouhou *et al.*, 2007). On the contrary, much higher PV values (204.5-342.3 meq. O<sub>2</sub>/kg oil) were also reported (Zzaman *et al.*, 2014). Variations in PV values could be related to the difference in the extraction method, source and nature of the seeds, difference in the period of seed storage before processing and also variation in the storage conditions, particularly humidity and temperature.

The stability of oils against oxidation during shelf life is one of the most important quality criteria. It can be evaluated in a rapid manner to save long storage time by using the accelerated oxidation test under high temperature which is known as the Rancimat test (Lutterodt *et al.*, 2010). Table 1 shows that SPO persisted much longer and possessed a higher oxidative stability index (40.07 h) in the Rancimat test

than HPO (0.51 h). This clearly indicates that the SPO had more inherent antioxidant agent(s) which enabled the oil to persist for longer against oxidation than HPO. The higher total phenolic contents of SPO (67.87 mg gallic acid/kg oil) could be one of the major reasons for its stability against oxidation, as indicated before. Previous studies have revealed the direct correlation between the total phenolic content and oxidative stability of *N. sativa* crude oil. For instance, an oxidative stability index of 55.0 h in the Rancimat test was reported for a crude oil of *N. sativa* that had total phenolic content 309.0 mg gallic acid/kg oil (Cheikh-Rouhou *et al.*, 2007).

Beside the crucial role of the total phenolics in suppressing the oxidation process and prolonging shelf life, the presence of a minor volatile oil fraction in the crude oil of *N. sativa* can also contribute to its oxidative stability. In the current investigation, that volatile oil fraction was separated from the crude oils of both SPO and HPO using hydrodistillation. A quantitative evaluation revealed that SPO contained significantly higher volatile oil content (2.6 wt % based on crude oil) than HPO (1.78 wt % based on crude oil). A study by Edris, (2011) revealed a variation in the oxidative stability index (26.9 h versus 2.58 h) of two different crude oils of *N. sativa* as a function of their volatile oil content (1.8 wt % versus 0.1 wt %), respectively. The importance of the volatile oil fraction in the suppression of oxidation of its parent crude oil is attributed to its content of thymoquinone (TQ). This volatile compound is known to possess a potent antioxidant activity (Ismail *et al.*, 2009). The results in Table 2 indicate that the thymoquinone (TQ) percentage of the volatile oil isolated from the SPO was higher (68.13%) than that isolated from the HPO (56.29%). This is due to the higher volatile oil content in SPO (2.6%), as previously indicated. In addition, TQ in its native state is composed of solid crystals with a melting point of 45–47 °C. So the heat generated during the extraction of SPO (~50 °C) can cause melting of the crystals of this compound and facilitate its co-extraction with the crude oil leading to a higher percentage in the volatile fraction of SPO. One should bear in mind that TQ is the compound that gives the volatile oil and consequently its parent crude oil their functionality due to the pharmaceutical and therapeutic properties of this compound (Gülşen *et al.*, 2016; Darakhshan *et al.*, 2015; Schneider-Stock *et al.*, 2014). From the above mentioned, it could be presumed that the high yield of the volatile oil fraction (2.6%) along with its high content of TQ (68.13%) of the SPO can be considered contributing factors that justify the high oxidative stability and relatively lower PV of that oil, as mentioned in the previous sections.

Table (2) also shows that p-cymene is the second most abundant constituent in the volatile oil fractions of SPO (20.11%) and HPO (26.2%). However, this

compound does not contribute to the oxidative stability of its parent crude oils as TQ does, due to the lack of proton donating groups in its chemical structure.

Color is one of the attributes of seed oils which originates from the presence of some pigments in the seeds or the plant part from which the oil is extracted. In case of *N. sativa*, the epidermis layer of the seeds' testa is composed of compact polygonal cells which are rich in dark-colored pigments. These pigments mainly include chlorophyll and carotenoids at different ratios which can reach up to 2.0 mg/kg each, depending on the seeds (Zzaman *et al.*, 2014). These pigments, especially chlorophylls and carotenoids, are co-extracted with the oil upon the expression of the seeds, giving the oil its characteristic color. In the current investigation, the authors studied the effect of expression techniques on the color characteristics of the obtained crude oil of *N. sativa*. HPO had lighter colors than screw pressed oil (SPO), which appear darker. This result is a common consequence of the high extracting ability of the screw pressing process.

The antimicrobial activity of the crude oil of *N. sativa* was previously addressed by different investigators particularly against multi-drug resistant bacteria (Salman *et al.*, 2008). However the effect of pressing technique on the extent of this activity is yet to be investigated. Therefore in the current investigation the antimicrobial activities of HPO and SPO of *N. sativa* against different pathogenic microorganisms were subjected to evaluation at two different doses, 20  $\mu$ l/well and 40  $\mu$ l/well. Table 3 shows that there is a differential behavior of antimicrobial activity between HPO and SPO. This behavior can be rationalized based on the difference in the content of the volatile oil fraction and TQ percentage in each of the two crude oils. In more detail, at 20  $\mu$ l/well, HPO contained 0.299 mg of the volatile oil fraction while SPO contained 0.437 mg, (based on the content of each volatile oil in both crude oils, see results section). Meanwhile, at the higher concentration (40  $\mu$ l/well) HPO contained 0.598 mg while SPO contained 0.874 mg of that volatile fraction. This volatile fraction and its major constituent TQ are the main cause of the antimicrobial activity of *N. sativa* crude oil (Chaieb *et al.*, 2011), and both of them were higher in SPO. One should also consider the potential participation of the other non volatile constituents like the phenolic compounds to the overall antimicrobial activity of the crude oils. These compounds were higher in the SPO than in HPO, as previously indicated (Table 1). Based on the above mentioned, it worth indicating that the high antimicrobial activity of SPO, which was illustrated in Table 3, can add to the functionality of that oil. Therefore, the consumption of SPO as a daily supplement may potentially protect against illness caused by pathogenic bacteria which contaminate minimally processed or unprocessed food products.

## 5. CONCLUSIONS

In conclusion, the screw pressing extraction of *N. sativa* seeds can produce a crude oil with better characteristics than hydraulic pressing under the conditions of the current investigation. This result could be of interest to the complementary supplements and nutraceutical field to take into consideration for maximizing the functional properties and general quality of *N. sativa* crude oil.

## Conflict of interest

The authors declare that there is no conflict of interest.

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