PRODUCTION OF COLD AND HOT PRESS OF *Nigella Sativa L* OIL AND MEAL USING SCREW PRESS

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Allah changed not the condition of a folk until they (first) change that which is ini their hearts. (Q.S. Ar-Ra'd: 11). Moreover, when it is said; come up higher! Go up higher; Allah will exalt those who believe among you, and those who have knowledge, to high ranks. Allah is informed of what you do. (Q.S. Al-Mujaadilah: 11)

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Success is not measured by what you accomplish,

However, the failure that you have faced and the courage

That makes you keep fighting against the obstacles that come insistently.

(Orison Swett Marden)

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LIST OF ABBREVIATION

N. sativa	*Nigella sativa L*
TPC	Total Phenolic Content
FAO	Food and Agriculture Organization
RM	Ringgit Malaysia
UPM	Universiti Putra Malaysia
NSM	*Nigella sativa* Meal
SBM	Soybean Meal
Ca	Calcium
K	Potassium
P	Phosphorous
B	Boron
Cu	Cuprum
Fe	Iron
Mn	Manganese
Mo	Molybdenum

Zn	Zinc
Cl	Chloride
Cd	Cadmium
Pb	Lead
Ni	Nickel
INQ	Index Nutritional Quality
M±SD	Mean ± Standard Deviation
HNE	Human Neutrophil Elastase
COPD	Chronic Obstructive Pulmonary Disease
ORR	Relative Rate of Oxidation
TQ	Thymoquinone
WHO	World Health Organization
TBA	2-Thiobarbituric acid
PV	Peroxide Value
SN	Saponification Number
FFA	Free Fatty acid
AV	Acid value

DPPH	1,1-diphenyl-2-hydrazyl
RSC	Radical Scavenging Capacity
GA	Gallic Acid
EAA	Essential Amino Acid
Non- EAA	Non-Essential Amino Acid
AA	Amino Acid
ANOVA	Analysis Variance
R8	Diameter of shaft screw with 8 mm
R11	Diameter of shaft screw with 11 mm
SFA	Saturated Fatty Acid
MUFA	Monounsaturated Fatty Acid
PUFA	Polyunsaturated Fatty Acid
CHO	Carbohydrate

PENGHASILAN MINYAK DAN SISA *Nigella Sativa L* SECARA TEKANAN SEJUK DAN PANAS MENGGUNAKAN SKRU MENEKAN

ABSTRAK

Kesan fizikal daripada skru mesin tekan terhadap hasil minyak biji *N. Sativa* dikaji menggunakan mesin ‘KOMET Screw Oil Expeller’. Saiz nozel yang berbeza (6, 10, dan 12 mm), kelajuan pengekstrakan (21, 54, 65 dan 98 rpm) dan diameter skru aci (8 dan 11 mm) diaplikasikan dalam ujikaji ini. Penghasilan minyak yang paling tinggi adalah ketikamana parameter-parameter ini digabungkan; skru aci dengan 8 mm diameter, kelajuan pusingan pada 21 rpm dan saiz nozel dicatatkan pada 6 mm. Penghasilan minyak paling tinggi ialah pada suhu 50 °C (tekanan sejuk) (22.68 %) dan paling rendah pada 100 °C (tekanan panas) (15.21 %). Peratusan hasil sisa benih *N. sativa* adalah lebih rendah pada 50 °C (73.48 %) dan lebih tinggi pada 100 °C (80.50 %). Hasil minyak dan sisa dengan ketara berbeza ($p<0.05$) dalam suhu yang berbeza. Fizikokimia dan ciri-ciri yang berkualiti daripada minyak biji *N. sativa* pada suhu tekanan sejuk dan panas (50 °C, 60 °C, 70 °C dan 80 °C, 90 °C, 100 °C) telah ditentukan. Keputusan yang diperolehi untuk suhu yang berbeza; FFA dan AV masing-masing, paling rendah pada 100 °C (0.19 % dan 0.39 %) dan tertinggi pada 80 °C (0.24 % dan 0.47 %); K_{232} malar untuk semua suhu antara 2.82-2.83 dan K_{270} adalah paling rendah pada 50 °C (1.48) dan tertinggi pada 100 °C (1.61); karotenoid pada 50 °C (1.95 mg/kg) adalah paling rendah berbanding dengan 100 °C (2.46 mg/kg); SN terendah pada 50 °C (132.75 mg KOH/g minyak) dan tertinggi pada 70 °C (198.21 mg KOH/g

minyak); kelikatan pada 60 °C (63,80 mPaS) adalah lebih rendah daripada sebanyak 100 °C (71.47 mPaS); nilai peroksida adalah tertinggi pada 80 °C (342 milisetara O_2/kg minyak) dan paling rendah pada 50 °C (204.58 milisetara O_2/kg minyak, klorofil adalah 1.97-2.50 mg/kg dan ketumpatan antara 0.93 sampai 0.98 g/cm^3. Warna daripada minyak dari warna hijau menjadi kekuningan dengan suhu yang semakin meningkat. Ahli panel analisis deria sederhana suka minyak ditekan pada 60 °C. Jumlah kandungan fenolik (TPC) minyak tertinggi pada 50 °C. Nilainya akan menurun dengan peningkatan suhu sehingga suhu 90 °C dengan sedikit peningkatan pada tanda 100 °C. TPC dalam benih sisa tidak jauh berbeza ($p<0.05$) dengan meningkat suhu. Kapasiti cara memerangkap radikal (RSC) benih minyak dan sisa adalah yang tertinggi (95.39% dan 91%) pada 50 °C dan paling rendah pada 100 °C (92.23% dan 68.53%). Nilai TBA minyak tidak jauh berbeza ($p<0.05$) dengan meningkat suhu. Tetapi pada suhu 80 °C adalah berbeza signifikan ($p<0.05$) dengan suhu 100 °C dalam biji sisa. Keputusan kajian ini menunjukkan bahawa pada suhu tekan sejuk, biji minyak dan sisa daripada *N. sativa* mempunyai sifat antioksidan yang tertinggi. Profil asid lemak, kandungan mineral, asid amino dan analisis hampiran minyak dan sisa benih *N. sativa* tidak jauh berbeza pada suhu pengekstrakan yang berbeza kecuali methionine, glysine, cysteine, kelembapan, dan kandungan abu.

PRODUCTION OF COLD AND HOT PRESS OF *Nigella Sativa L* OIL AND MEAL USING SCREW PRESSING

ABSTRACT

Effects of physical parameters of a screw press machine on oil yield of *N. sativa* seeds were studied using a KOMET Screw Oil Expeller. Different nozzle size (6, 10, and 12 mm), extraction speed (21, 54, 65 and 98 rpm) and diameter of shaft screw (8 and 11 mm) pressed at 60 °C were applied in this study. Highest percentage of oil yield recorded was at combination of shaft screw with diameter of 8 mm, rotational speed at 21 rpm and nozzle size of 6 mm. Oil yields were higher at 50 °C (cold press) (22.68 %) and lower at 100 °C (hot press) (15.21 %). Percentage meal yield of *N. sativa s*eeds were lower at 50 °C (73.48 %) and higher at 100 °C (80.50 %). The oil and meal yield were significantly different ($p<0.05$) in different temperatures.

Physicochemical and quality characteristics of *N. sativa* seed oil pressed at cold and hot press (50 °C, 60 °C, 70 °C and 80 °C, 90 °C, 100 °C) temperatures were determined. Results obtained for different temperatures; FFA and AV respectively, were lowest at 100°C (0.19% and 0.39%) and highest at 80°C (0.24% and 0.47%); K_{232} constant for all temperatures ranged from 2.82 to 2.83 and K_{270} was lowest at 50°C (1.48) and highest at 100°C (1.61); carotenoid at 50°C (1.95 mg/kg) was lowest compared to 100°C (2.46 mg/kg); SN was lowest at 50°C (132.75 mg KOH/g oil) and highest at 70°C (198.21 mg KOH/g oil); viscosity at 60°C (63.80 mPaS) was lower than that of 100°C (71.47 mPaS); peroxide value was highest at 80°C (342. meq O_2/kg oil) and lowest at 50°C (204.58 meq O_2/kg oil, chlorophyll ranged from 1.97 to 2.50 mg/kg and density ranged from 0.93 to 0.98 g/cm^3. The colour was from greenness to

yellowness with increasing temperatures. Sensory analysis panellists were liked moderately of oil pressed at 60° C.

Total phenolic content (TPC) of the oil is highest at 50°C. Its values decrease with an increase in temperature until a temperature of 90°C with a slight increase at the 100°C mark. TPC in the meal seeds was not significantly different ($p<0.05$) with increase temperatures. The radical scavenging capacity (RSC) of oil and meal seeds was highest (95.39% and 91%) at 50 °C and lowest at 100 °C (92.23% and 68.53 %). TBA value of oil was not significantly different ($p<0.05$) with increase temperature. But at temperature of 80 °C was significant different ($p<0.05$) with temperature of 100 °C in the meal seeds. The results of this study indicated that at temperature of cold press, the *N. sativa* seed oil and meal has the highest antioxidant properties. The fatty acid profile, mineral contents, amino acid and proximate analysis of oil and meal seeds of *N. sativa* were not significantly different at different extraction temperatures except for methionine, glysine, cysteine, moisture and ash content.

CHAPTER 1

INTRODUCTION

1.1 Background

Nigella *(Nigella sativa L)* known as black cumin is a typical of an annual herbaceous plant belonging to the *Ranunculaceae* family. These plants natively grow around the Mediterranean Sea region. The plantsmay stand at 16-24 inches tall, with light-coloured foliage and annual white-petaled flowers, yielding sharp-cornered rectangular seeds (no more than 3 mm long) and which exhibit carminative properties (Goreja, 2003, Ramadan, 2007). *Nigella sativa* is now cultivated from the Near East to India. The oilextracted from this seed was a precious remedy for the Egyptians and because of its benefits was called 'Pharaohs oil'. *N. sativa* seed oil has been a common oilseed found in supplements, food ingredients, medicine and cosmetics for centuries long. It was narrated by Abu Hurairah that Prophet Muhammad said to keep taking habbatussauda'*(N. sativa* seeds) because it could cure all diseases except death (Al-Muslim, Hadith no. 2215).

*N. sativa*seed oil contains antioxidant which has been proven to be beneficial for human health.*N. sativa* seed oil can be used as an antimicrobial agent (Ramadan, 2007), helps to rid the intestines of worms, has an anti-inflammatory impact (Mutabagani and El-Mahdy, 1997), possess anti-oxidant activity (Rahman and S, 1985, Rahman *et al.*, 1992, Ghosheh *et al.*, 1999, Badary *et al.*, 1999, Badary and El-Din, 2001) anti-tumour activity (Badary *et al.*, 1999), anti-cancer (Mabrouk *et al.*, 2002), exerts positive impact on immune system (Hailat *et al.*, 1995) and gastroprotective (El-Dakhakhny *et al.*, 2000, El-Abhar *et al*., 2003). Antioxidants are components that can neutralize free radicals created during the aging processing thus reducing health hazards, with a potential role in preventing some chronic diseases. It can also extend the shelf life of food products(Arranz *et al*., 2008). Houghton *et al*(1995) reported that thymoquinone, the main active constituent of volatile oils in *N. sativa* oil can inhibit peroxidation in ox brain phospholipids liposome. Ramadan(2007) has previously reported thatBurits & Bucar (2000)and Moos *et al* (2000) have studied the radical scavenging effect of thymol, thymoquinone and dithymoquinone on reactions that generate reactive oxygen species and singlet oxygen using the spectrophotometric methods which is to be applied in this study.

Common methods to extract oil from the seeds include either mechanical expression, solvent extraction, or a combination of both methods (Owolarafe *et al.*, 2003, Oyinlola *et al.*, 2004). Solvent extraction process is able to extract almost 90% oil but necessary equipments are costly, dangerous in correlation with fire and explosion, and the solvent usage calls the need for further processing (Yayock *et al*., 1988, Adeeko and Ajibola, 1990, Bamgboye and Adejumo, 2007).

Mechanical oil expression can be obtained using screw press by cold press or hot press. Mechanical screw pressing is the most popular method in the world to separate oil from vegetable oilseeds on a small to medium scale(Singh and Bargale, 2000). Oil yield from this method was often higher in quality compared to solvent-extraction method as determined by some attributes such as higher oxidative stability and lower non hydratable phospholipids (Said, 1998, Wiesenborn *et al*., 2001).Atta (2003) found that cold-pressed crude oil is more stable to auto-oxidation rancidity compared to the crude oil resulting from solvent extraction with significant difference in physicochemical properties of oil. But the screw presses does not have high extraction efficiencies and seldom exceed 80% while solvent extraction achieves over 98% (Srikantha, 1980, Bargale, 1997).

One type of the screw press is the KOMET DD 85-1G oil expeller which produced 5- 10 kg/h with a single conveying screw instead of individual compression screws, to squeeze the oils from various oil bearing seeds. A further advantage of this system is that virtually all oil bearing seeds, nuts and kernels can be pressed with the standard equipment without cumbersome adjustment of screws and oil outlet holes. Another advantage of this system is that easy to maintain, the press cake extrudes out of the oil press in the shape of pellets that are easy to store and handle. The quality of oilseed from this machine which can be used for human consumption without any conditioning depends on the raw material (http://www.oekotec.ibg-monforts.de). But different accessories of this machine and raw material will probably generate seed oils with different capacity and characters. Some researchers have been reported that the parameters before and during pressing will affect the oil pressing processes including particle size, heating temperature, heating time, moisture content, and applied pressure (Adeeko and Ajibola, 1990, Olayanju *et al*., 2006, Baryeh, 2001, Mwithiga *et al*., 2007).

Lee *et al*(2004)found that there was a significant difference of the physicochemical properties of Safflower seeds oil with different pressing extractiontemperature. The studies showed that there was a variation in physicochemical of safflower seed oil with increasing of temperature and even so with oxidative stability of oils, but there was no significant difference in fatty acid composition. High temperature can accelerate oxidation process in oil seed (Choe and Min, 2006). Xu *et al*(1999) stated that the increase in the heating temperatures to above 100°C could cause oil break down, producing peroxides, acids and other free radicals while decreasing its total phenolic content (TPC).

According to Food and Agriculture Organization (FAO), Malaysia is the 4th largest oilseeds producing country in the world with a total production of 150000 metric ton (MT)(FAOSTAT, 2008). Malaysia is also the base for Asia Pacific seacoast and expenditures for manufacturing health products of *N. sativa* seeds. There are about 20 products that have been marketed and consumed in the form of tea, oil, honey, shampoo, soap, skin cream, original oil, “winter oil” and many more (www.utusan.com.my). Prof. Dr. Maznah Ismail from Universiti Putra Malaysia (UPM) also said that there are three researchers studying the extraction N. *sativa* seeds as nutraceutical, antioxidant and supplement. This project is being supported by a grant from UPM with an amount of RM 283,000.00 (www.utusan.com.my).

The significant of this study is to give information about the selection for the best processing method to obtain *N. sativa* seeds oil. There is a lack of study on the effects of temperature on its yield and quality in terms of physicochemical characteristics and antioxidant properties of *N. sativa* seeds oil and meal. The processing method selection can help to gain a good quality of *N. sativa* seeds oil. The data obtained is expected can assist the producer to select the best method which suitable according to their budget.

1.2 Objectives

The overall objectives of this research is to study the effects of physical parameters of the screw press machine on oil yields, effects of extraction temperatures of screw press at different temperatures (cold and hot press) on yields and quality and characteristics of oil and meal from *N. sativa* seeds. More specifically, the objective of the research is:

1. To study the effects of nozzle sizes, shaft screw and speed of the screw press machine at 60 ºC on oil yield from *N. sativa* seeds.
2. To study the effects of extraction temperatures (50 °C-100 °C) on the yield, physicochemical, sensorial and antioxidant properties of *N. sativa* seeds oil and meal.

CHAPTER 2

LITERATURE REVIEW

2.1 *Nigella sativa L*

Nigella sativa L is from genus *Nigella L* of the family Ranunculaceae (Al Gaby, 1998, Cheikh-Rouhou *et al.*, 2007) which originated from Mediterranean Sea (Gad *et al.*, 1963) (Table 2.1). Over the years, its growth spread throughout northern Africa, eastern Asia, and southern Europe, and in the past few decades, as immigration helped the plant's cultivation and popularity to spread even more extensively. Nowadays, *N. sativa* has been growth in Middle East and Mediteranean Basin mainly in India, Bangladesh, Egypt, the Sudan, Turkey, Iraq, Iran and Pakistan (Goreja, 2003).*N. sativa*thus developed into another common terms such as what comes from Saudi Arabia "Habbet el Baraka" and "Shunez" (Burits and Bucar, 2000) from Romania and Russia "Charnuska", in India and Pakistan known as "Kalonji". *N. sativa* plant has white and blue petals with 18 inches of tall, with bright-coloured leaves. It has black flower seeds with a strong odour, spicy flavour similar toanise or nutmeg. These seed was called "Jinten hitam" in Indonesia and "Jintan hitam"in Malaysia. *N. sativa* flower blossoms in July and the seeds ripen in September. The flowers are hermaphrodite (have both male and female organs) and are pollinated by bees. The plant prefers light (sandy), medium (loamy) and heavy (clay) well-drained soils that are acid, neutral and basic (alkaline). The form and bulk of this seeds is small, triangular and thin similar with black sesame seeds but the texture more hard and rough (Figure 2.1).*N. sativa* cannot grow in the shade and requires dry or moist soil (Abdel Moneim *et al.*, 1997).

Table 2.1 Scientific classification of *N. sativa* seeds

Scientific classification	
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Magnoliidae
Order	Ranunculales
Family	Ranunculaceae
Subfamily	Ranunculoideae
Species	Nigella sativa L
Genus	Nigella L
Tribe	Nigelleae

(http://www.plants.usda.go)

Figure 2.1*Nigella sativa L* seeds

In Egypt, people used to use *N. sativa* seeds for external uses such as to protect the skin and also to aid digestion. Efficiency of the seeds was first written in the *Old Testament of the Bible* (Isaiah 28: 25-27) and the Prophet Muhammad also said that "*these seeds can cure all diseases except death*" (Al-Muslim, Hadith no. 2215).

In Persia, *N. sativa* seeds were introduced by a famous scholar and physician, Ibn Sina through his treatment then he wrote this experience in the book of *Al-shifa'*. He said that the seeds stimulates the energy of the body and helps recovery from fatigue or sadness. These seeds have ability to detoxify, anti-bilial effects which can heal respiratory dysfunction(Wu *et al.*, 1999). He also recommended this herb for many other diseases and could be consumed by children.

In Turkey, these seeds are usually used as a mullets or magic antidote sciences. In the east hemisphere such as India, these seeds are also used as medicine and as stimulant. In Asia, these seeds were found in everywhere whether in the market or even at home(Goreja, 2003)

In Europe, these seeds were first discovered by a scientist from Rome, Pliny the elder in the Naturalis Historica, the world's first encyclopedia of natural science. In medieval times, Dioscorides was described *Nigella* seeds in his treatment and healing book. Since then, the name of *Nigella* change becomes *black seed*. In the 18^{th} century, there was misunderstood efficacy of black seed for many years by Germany who said that the seeds are weed and contain danger toxic. However, the efficacy of these seeds was come again after veterinarians from Egypt who stayed in Europe, made research using these seeds. These seeds were mixed in the animal food for curing animals in Europe. This treatment was successfully and attracted other researcher from German to observe these seeds in their project (Luetjohann, 1998, Goreja, 2003). Few Western manufactures have been able to control the multitude of variables involved in growing plants that yield healthy and potent *N. sativa* seed oil.

2.2 Compositions of*N. sativa* seeds

Oilseeds were extracted from *N. sativa* seeds for produce oils (32-40 %) and residual meal that rich of macronutrients (protein, fibre, mineral), micronutrients (vitamin and minerals) and antioxidants. Meal seeds were used mainly for feeding animal to lesser extent for human food and as a fertilizer (Zeweil *et al.*, 2008, Abdel-Aal and Attia, 1993, El-Fatatry, 1975, Rossell and Pritchard, 1991).

2.2.1 Macronutrient

Macronutrients are *nutrients that provide calories or energy for growth, metabolism, for other body functions*, e.g. protein, carbohydrate, fat which needed in large amounts (www.mckinley.illinois.edu). The *N. sativa* seeds contains of both fixed oil and volatile oils, proteins, alkaloids and saponin (Burits and Bucar, 2000, Abu-Zinadah, 2009). The composition are fixed oil (32-40%), volatile oil (0.45%), protein (20%), minerals (3.74%), carbohydrates (34%), fibres (5.50%) and water (6%) (Aggarwal and Kunnumakkara, 2009).

The fixed oil in *N. sativa* seeds oils arepalmitic, stearic and myristic acid (saturated fatty acids); as well as arachidonic, eicosadienoic, linoleic, linolenicand oleic acid (unsaturated fatty acids)(Burits and Bucar, 2000).*N. sativa* seeds oil provides rich supply of polyunsaturated fatty acids. Three polyunsaturated acids are absolutely necessary for cellular growth in all mammals. These are arachidonic, linoleic, and linolenic acids. The body supplies each of these except linolenic acid, which must be obtained through outside sources (Goreja, 2003).

Table 2.2 Proximate contents in *N. sativa* seeds

Proximate analysis	**Value of analysis (%)**	**References**
Crude protein	22.75	Al-Beitawi and El-Ghousein, 2008
	20-27	Abdel-Aal and Attia, 1993; Salem, 2001; Takruri and Dameh, 1998
	26.7	Cheikh_Rouhou *et al*, 2007
	22.80	Sultan, 2009
Moisture	4.40	(Al-Beitawi and El-Ghousein, 2008)
	5.52-7.43	(Abdel-Aal and Attia, 1993; Salem, 2001; Takruri and Dameh, 1998)
	8.65	(Cheikh_Rouhou *et al*, 2007)
	6.46	(Sultan, 2009)
Ash	4.45	(Al-Beitawi and El-Ghousein, 2008)
	3.77-4.92	(Abdel-Aal and Attia, 1993; Salem, 2001; Takruri and Dameh, 1998)
	4.86	(Cheikh_Rouhou *et al*, 2007)
	4.20	(Sultan, 2009)
Crude oil	30-38	(Edris, 2009)
	30-35	(Dandik and Aksoy, 1992)
	34.49-38.72	(Abdel-Aal and Attia, 1993; Salem, 2001; Takruri and Dameh, 1998)
	28.48	(Cheikh_Rouhou *et al*, 2007)
	36.25	(Al-Beitawi and El-Ghousein, 2008)
	31.16	(Sultan, 2009)
Carbohydrate	23.5-33.2	(Abdel-Aal and Attia, 1993; Salem, 2001; Takruri and Dameh, 1998)
	40	(Cheikh_Rouhou et al, 2007)

.

The macro minerals in the *N. sativa* seeds are calcium, phosphorous, potassium, magnesiumand sulphur.Takruri & Dameh (1998) have conducted research on the mineral contents and proximate analysis of *N. sativa* seeds from five different sources (Indian, Jordanian, Syirian 1, Syirian 2 and Turkish). They found that Indian seeds were have higher minerals contains e.g. K (5517 mg/kg), Ca (1932 mg /kg), P (5043 mg/kg) and crude protein (241 mg/kg).

Amino acids in *N. sativa* seeds are arginine, glutamate, leucine, lysine, methionine, tyrosine, proline and threonine. Five from eight amino acids in *N. sativa* are essential amino acid (Goreja, 2003).Table 2.2 shows the function of essential amino acid in *N. sativa* seeds.Cysteine, tyrosine, histidine and arginine were considered as semi essential amino acids in children, because the metabolic pathways that synthesize these amino acids are not fully developed (Wirth *et al.*, 1978).

Alkaloids that were found in *N. sativa* seeds belonging to two different types: isoquinoline alkaloid is represented by is nigellimine and nigellimine-N-oxide and pyrazol; includes nigellidine and nigellicine (Nickavar *et al*., 2003). Nigellimine as 1-methyl-6,7-dimethoxy isoquinoline as well as by its conversation to nigellimine-N-oxide (Rahman *et al.*, 1992). While many alkaloids are poisonous, some are used medicinally as analgesics (pain relievers) or anaesthetics, particularly morphine and codeine, and for other uses.

N. sativa seeds contain beta-sterol which supports secretion throughout the body and agent for lowering cholesterol. The main components of phytosterols in *N. sativa* seeds oil are β-sitosterol and stigmaterol (65 % of total sterols) followed Δ5-avenasterol (1.025g/kg), Δ7-avenasterol (0.809 g/kg) (Cheikh-Rouhou *et al.*, 2008). Prostaglandin E1 also contributes to *N. sativa* seeds general balancing action by maintaining the salt and water balance, and regulates insulin secretion, nerve conduction, and gastrointestinal function (Goreja, 2003).

Table 2.3The function of essential amino acid content of *N. sativa* seeds

Essential	**Function**
Isoleucine	Provide ingredients for the manufacturing of other essential biochemical components in the body.
Leucine	Helps for production energy, stimulants to the upper brain.
Lysine	It aids in the production of antibodies, hormones and enzymes. Effective against herpes by improving the balance of nutrients
Methionine	Helps for lower cholesterol lever, prevents disorders of the hair, skin and nail.
Phenylalanine	Reduces hunger pains, improve memory because help to produce norepinephrine and antidepressant.
Threonine	Important constituent of collagen, elastin and enamel protein. Helps the digestive and intestinal tracts function more smoothly.
Tryptophan	Reduce anxiety, depression, and the risk of artery, heart spasms and helps in the treatment of migraine headaches. It helps alleviate insomnia.
Valine	Promotes mental vigor, muscle co-ordination and calms emotions
Arginine*	Improved immune responses to bacteris, viruses and tumour cells.

(*) non essential amino acid (Ramadan, 2007); www.balckseed-hemp-oil.org , 2007)
N. sativa seed oil contained significantly amounts of important mineral elements.

The main trace elements are phosphorous, calcium, magnesium and iron. Potassium is the most abundant element in the black cumin seeds, followed by phosphorous and calcium (Cheikh-Rouhou *et al*., 2007).

2.2.2 Micronutrients

Micronutrients are minerals and vitamin which required in much smaller amounts than those of the primary nutrients e.g. nitrogen, phosphorus and potassium used for plant growth. The micronutrients were included of boron (B), copper (Cu), and iron (Fe), manganese (Mn), molybdenum (Mo), zinc (Zn), chloride (Cl), B1, B6, niacin and folic acid of *N. sativa* seeds from another source (Table 2.4). Takruri and Dameh (1998) stated that micronutrients content in *N. sativa* seeds are good source of protein, many vitamins and minerals according to index nutritional quality (INQ). There were reported presence of sodium, iron, zinc, and copper in quantities 496, 105, 60.4 and 18.4 mg/kg, respectively (Ashraf *et al.*, 2006, Cheikh-Rouhou *et al.*, 2007). *N. sativa* seeds from India have higher micronutrients composition from other source seeds. An *N. sativa seed has* strength to enhance of women fertility because it has high of folic acid (Table 2.4). Al-Naqeep *et al*(2009) reported that *N. sativa* seeds from Yemen high amount of calcium, potassium, magnesium, phosphorous and iron about 811, 563, 234, 77.40, 80.70 and 55.60 g/100 g, respectively.

Table 2.4 Micronutrient of *N. sativa* seeds from different sources (mg/kg)

Seeds sources[a]	**Indian**	**Jordanian**	**Syrian 1**	**Syrian 2**	**Turkish**	**M±SD**
Mineral						
Fe	102	107	93	91	130	105±15.6
Cu	24	18	17	15	18	18.4±3.4
Na	550	419	535	5535	440	496±61.3
Zn	62	59	59	66	56	60.4±3.8
B1	13	14	13	18	15	14.6±2.1
B6	4	15	4	6	4	6.6±4.8
Niacin	48	33	97	nd	48	56.5±27.9
Folic acid	700	400	870	630	470	614±186.9

[a](Takruri and Dameh, 1998)

2.2.3 Antioxidant

Four components of *N. sativa* seeds, oil and meal namely, thymoquinone, carvacrol, t-anethole and 4-terpineol demonstrated respectable radical scavenging (antioxidant) property when tested in the phenylpicrylhydracyl assay for non-specific hydrogen atom or (Watkins, 2007).

2.2.3.1 Thymoquinone (TQ)

N. sativa extracts and some of its active principles, like thymoquinone (Figure 2.2), have been shown to inhibit non-enzymatic peroxidation in ox brain phospolipid liposome (Houghton *et al.*, 1995). *N. sativa* along with cystein, vitamin E and Crocus sativus protected cisplatin – induced haematological, hepatic and renal toxicities (El-Daly, 1998). Thymoquinone has also been reported to be active against *Aspergillus* *niger*, a common opportunistic saprophyte, which can infect AIDS patients and other immune deficient individuals (Al-Jabre *et al.*, 2003).

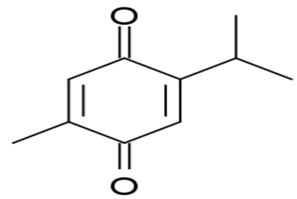

Figure 2.2 Chemical structure of Thymoquinone

Thymoquinone, an active principle of *N. sativa*, effectively inhibited the growth of *Fusarium solani* that is generally resistant to most anti-fungal drugs including capsofungin and newer azoles. The inhibitory effect of *N. sativa* seeds oil on *Listeria monocytogenes* was twice more than that of gentamicin. (Nair *et al.*, 2005)

2.2.3.2 Carvacrol

The volatile oil component in *N. sativa* seeds, which has a hydroxyl group in position 3 on the benzene ring, is the most bioactive molecule in inhibiting human neutrophil elastase (HNE) activity. Inhibition of HNE activity by carvacrol was explained by its direct binding with the enzyme, forming an enzyme – inhibitor complex. Carvacrol can be considered a natural anti elastase agent in the treatment of injury in chronic obstructive pulmonary disease (COPD) and emphysema (Kacem and Meraihi, 2006).

2.2.3.3 Tocopherol

Tocopherol or vitamin E is a fat – soluble vitamin that is an important antioxidant.

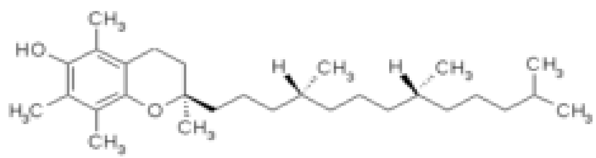

Figure 2.3 Chemical structure of α-Tocopherol

α-Tocopherols has been shown to have a pro-oxidative action at concentrations greater than 0.01% during early autoxidation stage in lard, corn, and olive oil triacylglycerols and the most important of minor components in oil and fats (Figure.2.3) (Chimi *et al.*, 1991, Wei *et al.*, 1995, Blekas *et al.*, 1995). Tocopherols are very stable with respect to heat and have an excellent carry-through effect, more effective in increasing the oxidative stability and in reducing the relative rate of oxidation (ORR) with increasing temperature(Schmidt and Pokorny, 2005).

2.2.3.4 Carotenoids

Carotenes are hydrocarbons built from isoprene units; xanthophylls are their oxygenated derivatives. β-carotene comes from one of conjugated double bonds of carotene in food(Moss and Weedon, 1976).

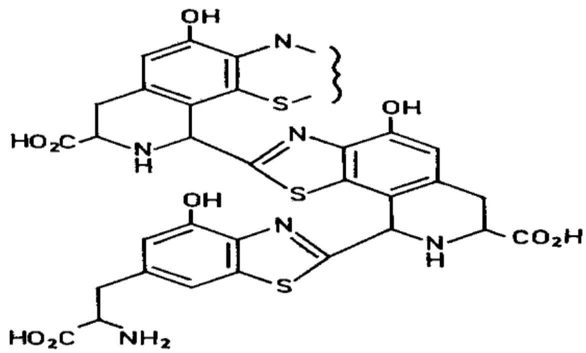

Figure 2.4 Chemical structure of carotenoid

Carotenoids are susceptible to oxidation because they are highly unsaturated and dispersion in food usually afford them protection against heat processing (Martin, 1960, Kiokias and Gordon, 2003)

2.3 Application of *N. sativa*

2.3.1 Medicinal

N. sativa seeds have been traditionally used as a carminative, diuretic, lactagogue and vermifuge. This seeds were also used for asthma, cough, bronchitis, headache, rheumatism, fever, influenza and hypertension (Hegnauer and Hohl, 1973, Burits and Bucar, 2000). Zaoui, *et al*(2002) stated that fixed oil from *N. sativa* seeds can decrease body weight of rats and is effective in controlling blood homeostasis in rats for chronic treatment. *N. sativa* seeds may also increase serum total protein (Al Gaby, 1998), significantly diminishes plasma glucose level to normal and alloxan-induced diabetic rabbits (Al-Hader *et al*., 1993). Extract of *N. sativa* was used as protector in mice and rats from cis-platin-induced falls in leukocytes counts, increased the haemoglobin levels, mean osmotic fragility and haematocrit (Clavel *et al*., 1991, El-Daly, 1998, Nair *et al*., 2005).

The thymoquinone and extract of *N. sativa* seeds have antidermatophyte activity against four species of *Trichophytonrubrum*, and one of each of *Trichophyton interdigitale, Trichophyton mentagrophytes, Epidermophyton floccosum* and *Microsporum canis*. This extract were also used for folk medicine e.g. for the treatments of fungal skin infections (Abu-Al-Basal, 2009) where it produced antinociceptive effects through indirect activation of the supraspiral µ1 and k-opioid receptor subtypes (Abdel-Fattah *et al*., 2000). These seeds also used as protective agents against the chromosomal aberrations induced as a result of schistosomiasis on mouse cells. El-Bahai *et al*(2009) stated that *N. sativa* seed as supplements can enhance levels of baseline peak tension, maximum rate of tension development heart rate and myocardial flow rate in rats. *N. sativa* seeds which were mixed with bee can be good for respiratory health, stomach and intestinal health, kidney, bladder and liver function, circulatory and immune system support and for general overall well-being (Handa *et al.*, 1998).

2.3.2 Nutritional

The *N. sativa* seeds have been used for soups, breads and pastries (Sultan *et al.*, 2009). *N. sativa* seed not only heightens the flavour of food, but aids in the well digestion process. Additionally, preferred for its milder flavour, *N. sativa* seed is often ground and used as a substitute for black pepper. A flavourful tea may be enjoyed by simply crushing whole *N. sativa* seeds and steeping into hot, though not boiling water (Goreja, 2003).

Whole seeds can be spindle into salads and various dishes a like sunflower or sesame seeds. Baked goods were topped with whole seeds or the seeds may be grounded and baked into the food. *N. sativa* seed also makes a savoury addition to meat dishes, especially pates and ground meat mixtures. This herb was used to prevented excessive oxidation (Leka, 1999, Sultan, 2009). *N. sativa* seeds was increased significantly the production of eggs, eggs mass, egg-shell thickness. As well as reduce the yolk cholesterol contents, low density lipoprotein and high- density lipoprotein cholesterol on poultry egg and suitable for human consumption (Akhtar *et al*., 2003).

N. sativa seeds meal (NSM) can be used as a protein-rich-meal for animal feed and fertilizer for plants. Zeweil, Ahmed, El-Adawy, & Zaki (2008) stated that NSM can be used to replace the imported soybean meal (SBM) and reduce the cost of rabbits feeding. They recommended to use NSM as a rabbits feed as a non-traditional source of plant protein without harmful effects on its growth performance, kidney or liver function. NSM could be additive ingredients in formulation feed-diet for growing lambs without any adverse effects, reducing feed costs and importantly increasing economic efficiency of industries (Abdel-Magid *et al*., 2007, El-Ayek *et al*., 1999).

2.3.3 Cosmetics

Since the age of King Tut, *N. sativa* seed has been became an essential beauty component for the people of many cultures. The legendary bronze complexion of ancient Egyptian royalty is commonly attributed to the use *N. sativa* seed in their daily skin care (Schleicher and Saleh, 2000). In the field of cosmetics usually *N. sativa* seeds are used as the main and additive ingredients mixed with honey, other herb and organic materials. The products of *N. sativa* seeds oil in cosmetics e.g. acne soap, shampoo, body lotion, cream for smooth skin and many others.

The essential oil of *N. sativa* seeds makes a great facial cleanser, and they contain of essential fatty acids make it become a fantastic moisturizer for hair and as well as for skin. It also fortifies the scalp, strengthens hair follicles, and promotes healthy nails and teeth. Furthermore, *N. sativa* seeds become an important component in invigorating mouth wash. It cleanses the mouth, reduces any swelling of the gums, and kills harmful germs and bacteria trapped between the gums and teeth (Goreja, 2003).

2.4 Quality of *N. sativa* seeds oil

2.4.1 Fixed oil

N. sativa seeds have highest linoleic and oleic acids (unsaturated fatty acids) with amounts found in the ranges of 47.0-60.0 and 18.9-25.69%, respectively. These seeds have lowest quantities of linolenic, arachidic and eicosenoic acids (Atta, 2003, Ashraf *et al.*, 2006, Cheikh-Rouhou *et al.*, 2007). Polyunsaturated fatty acids constitute the bulk of the oil with quantities ranging from 48-70%, while monounsaturated and saturated fatty acids are comparatively in lesser proportions (Üstun *et al.*, 1990, Nickavar *et al.*, 2003, Ramadan and Mörsel, 2003, Atta, 2003).

The palmitic, oleic and linoleic acid as the higher composition in *N. sativa* seeds oil (Table 2.3).Ramadan & Mörsel (2003)stated that composition of fatty acid in *N. sativa* seeds oil and Niger seeds oil have similar and higher than coriander seeds oil. Their found that the major saturated acids was namely palmitic and stearic acid; unsaturated fatty acids are oleic and linoleic acid as the main FAME in the *N. sativa*, coriander and niger seeds oil.

The difference source of *N. sativa* seeds, methods of oil extraction and treatments that were conducted on the seeds will affect the composition of the fixed, and volatile oil in the oil as indicated in Table 2.5. Solvent extraction method will be influenced by the used type of solvent, heating temperature, duration of extraction, and particle size of the samples (Oyinlola *et al.*, 2004). Atta (2003) was extracted the *N. sativa* seeds oil by using cold press and stated that fatty acid composition which using cold press is more stable to auto-oxidation rancidity compare to which using solvent extraction.

Table 2.5 Fatty acid composition of the fixed oil of *N. sativa* seeds oil

Fatty acid (%)	**Nickavar, *et al* (%)**	**Cheikh-Rouhou, *et al* (g/100 g)**	**Sultan (%)**	**Atta (%)**
Lauric acid	0.6	-	-	-
Myristic acid	0.5	0.35	0.42	11.1
Palmitic acid	12.5	17.2	12.07	12.1
Stearic acid	3.4	2.84	2.35	3.7
Oleic acid	23.4	25.0	19.65	18.9
Linoleic acid	55.6	50.31	57.38	47.5
Linolenic acid	0.4	0.34	1.13	2.1
Eicosadienoic acid	3.1	0.32	1.47	-
Arachidoni acid	-	0.14	0.33	1.2
Behanic acid	-	1.98	0.19	0.9

(Nickavar *et al.*, 2003), (Cheikh-Rouhou *et al.*, 2007),(Sultan, 2009),(Atta, 2003)

2.4.2 Volatile oil

Volatile oil found in *N. sativa* seeds are the originally isolated nigellone and thymoquinone (TQ) and was first discovered as aherb in 1985 (Gali-Muhtasib *et al.*, 2006). Nigellone offers both anti-spasmodic and bronchodilating properties, contributing to *N. sativa* seeds potency against respiratory ailments. Additionally, nigellone works as an antihistamine, thereby reducing the negative symptoms of allergy sufferers.

Thymoquinone displays amazing anti-inflammatory and analgesic properties. It is also a strong anti-oxidant and helps to cleanse the body of toxins (Daba and Abdel-Rahman, 1998, Chakravarty, 1993). The volatile oil in *N. sativa* seeds oil has been contained TQ (18.4-24%) and the monoterpens: pcymene 31.7% and α-pinene 9.3% wt/wt (El-Dakhakhny, 1963, El Tahir and Ageel, 1994). The thymoquinone, t-Anethole, carvacrol, p-cymene and limonene are main compound in *N sativa* seeds oil (Table 2.4). The actions of pharmacological compounds of TQ, p-cymene and á-pinene can act as cytoprotective agents against oxidative damage induced by free radical generatingagents (Mansour *et al.*, 2001, Badary *et al.*, 1998). Wajs *et al*(2008) stated that ρ-cymene as a major component of *N. sativa* seeds essential oil.

Table 2.6 Volatile oil of *N. sativa* seed oil

Compound	**a (%)**	**b (%)**	**c (%)**	**d (%)**
α-Pinene	1.2	0.30	0.20	1.48
Camphene	-	<0.01	-	-
β-Pinene	1.3	0.10	0.15	1.72
Sabinene	1.4	0.09	0.08	-
β-Myrcene	0.4	0.16	-	-
α-Terpinene	-	0.11	0.05	-
Limonene	4.3	0.29	0.37	-
β-Phellandrene	0.6	0.08	-	-
1.8-Cineole		0.04	0.19	-
γ-Terpinene	0.5	0.12	-	-
p-Cymene	14.8	12.0	7.07	32.02
α-Terpinolene	-	<0.01	<0.01	-
2-Heptanal	-	-	0.38	-
Thujene	2.40	0.07	0.15	2.40
Trans-Sabinenehydrate		0.12	0.12	-
Longipinene	0.3	0.21	0.20	-
Camphor	-	0.06	0.05	-
Linalool	-	0.25	0.44	-
Cis-Sabinenehydrate	-	0.09	-	-
Longifolene	0.7	1.26	1.31	-
Bornylacetate	-	0.28	0.49	-
2-Undecanone	-	-	-	-
4-Terpineol	0.7	4.02	3.43	-
Borneol	-	<0.01	0.24	-
Carvone	0.3	0.88	0.13	-
Thymoquinone	0.6	46.60	35.30	23.25
2-Tridecanone	-	0.11	0.22	-
t-Anethole	38.3	1.30	1.28	2.10
p-Cymene-8-ol	0.4	0.78	-	-
p-Anisaldehyde	1.7	-	-	-
Thymol	-	0.25	0.20	2.32
Carvacrol	1.6	11.6	8.50	10.38

a, b: *N. sativa* from Iran and Graz and Vienna (Nickavar *et al.*, 2003), **c**: *N. sativa* from extraction commercial, (Burits and Bucar, 2000), **d**:(Buettner, 1993, Sultan, 2009).

2.4.3 Physical characteristics of *N. sativa* seeds oil

The commonly physical analysis of oilseeds are colour (divided to nature colour and degradation of nature colour), odour, flavour, melting point, solubility, boiling point, softening point, density, viscosity, scavenging activity ($K_{232,}$ K_{270}), turbidity point and refractive index (Ketaren, 1986). The physical analysis of *N. sativa* seeds oil were density (0.91- 0.92 g/cm^3), viscosity (6.08 mPas), refractive index (1.467- 1.473), K_{232} (0.72-1.32), K_{270} (0.30-0.39)and colour L* (32.62), a* (2.25), b* (56.26), respectively (Atta, 2003, Mansuri *et al.*, 2011, Sultan, 2009).

Viscosity in vegetable oil was correlated with acyl chain length and degree of unsaturation. Cause of that, the high of unsaturation bond and fatty acyl chains, the viscous in the oil was high and temperature has effect to viscous of oil (Gunstone, 2002). Refractive index was related to molecular weight, fatty acid chain length, degree of unsaturation and degree of conjugation of oil. It was increased with rise of temperature (Erickson, 1995)

2.4.4 Chemical characteristics of *N. sativa* seeds oil

The WHO stated that dietary fat must be rich in polyunsaturated fatty acid more than 33% and reduced contents of saturated fatty acids less than 33% to health consumption. *N. sativa* seeds oil has rich in unsaturated fatty acids; linoleic and oleic acids were dominant in the ranges 47.0-60.0 and 18.9- 25.69 % and minor quantities of linolenic, arachidic and eicosenoic acids (Cheikh-Rouhou *et al.*, 2007, Atta, 2003, Ramadan and Mörsel, 2003). Clydesdale and Francis (1968) said there can be no significant chlorophyll gain on long term storage with heat processing. The mechanism of fading of the colour in chlorophyll is oxidative and probably involves attack on the C_{10} β-keto-ester group, eventually leading to low molecular weight, colourless compounds (Bassi *et al.*, 1985). The chemical analysis of *N. sativa* seeds oil showed in Table 2.7.