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Food application of *Nigella Sativa* essential oil

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Abstract:

Nigella Sativa Oil (NSO), extracted from seeds of *Nigella Sativa*, are widely used in traditional medicine and foodstuff in around the world because they are rich in a variety of phytochemicals. *Nigella sativa* essential oil (NSEO) are regarded as a promising trend to get more functional benefits from NSO, which can be obtained by distillation methods. Thymoquinone, thymohydroquinone, *p*-cymene, carvacrol and *t*-anethole are the main components of NSEO. These active compounds, especially thymoquinone, showed antioxidants, antibacterial and anti-inflammatory activities with low toxicity, which have been considered a useful source of food application. NSEO are, nowadays, still used in food in limited forms due to their low water solubility, high volatility and strong organoleptic properties; However, encapsulation technology could tackle these challenges. Thus, the ambition of this chapter is to provide an overview of NSEO and their advantageous properties usage on food application. Furthermore, biological activities of NSEO and encapsulated NSEO in addition to its technological and potential utilization in food will be presented.

Keywords: *Nigella Sativa*; *Nigella Sativa* Essential oil; Thymoquinone; Food application; Encapsulation;

32 1. Introduction

33 There has been an increasing interest in the use of unconventional oil seeds because
34 their ingredients have unique properties that may increase the supply of new products
35 in food application. In recent years, people have paid more attention to food safety, and
36 the demand for “natural” food additives has received increasing attention. The current
37 research focus on plant essential oil as a natural source of antibacterial and antioxidant
38 compounds. *Nigella sativa* is well known as black seed or black cumin, which belong
39 to the botanical family of Ranunculaceae ([Aggarwal and Kunnumakkara, 2009](#)). It is
40 an annual plant from Southwest Asia, North Africa, and Southern Europe. In addition,
41 *Nigella sativa* oil (NSO) has been used as a food additives or medicine improve
42 appearance and taste of products, and to treat fever, headache, anxiety, diarrhea, asthma,
43 and stroke throughout the history, owing to different complex chemical substances
44 ([Walton et al., 1999](#)). Different pharmacological trials have been carried out to consider
45 its constituents. NSO also contains a small amount of volatile oil (NSEO), which has
46 functional properties due to its active compounds: thymoquinone, thymohydroquinone,
47 thymol, carvacrol and *p*-cymene (Fig.1) ([Mariod et al., 2009](#); [Kanter, 2008](#)). Due to
48 these compounds, recently, many biological properties of *Nigella sativa* seeds and its
49 oils have been investigated, such as antibacterial ([Ferdous et al., 1992](#)), antioxidant
50 ([Mariod et al., 2009](#)), antitumor ([Worthen et al., 1998](#)), hyperglycemia and
51 hypercholesterolemia ([Sultan et al., 2009](#)). In addition, NSEO are regarded as a
52 promising trend to get more functional benefits from *Nigella sativa*, which can be
53 obtained by vapor or hydro distillation methods. However, the use of NSEO in food
54 application still have some limitations due to their low efficacy as food additives mixing
55 with other medias, their low solubility and their instability. Effective encapsulation
56 systems are considered to tackle these challenges in a certain degree. Thus, the purpose
57 of this chapter is to present and discuss the chemical composition, and biological
58 properties of NSEO, in order to explore their valuable uses for the development of food
59 industry.

60

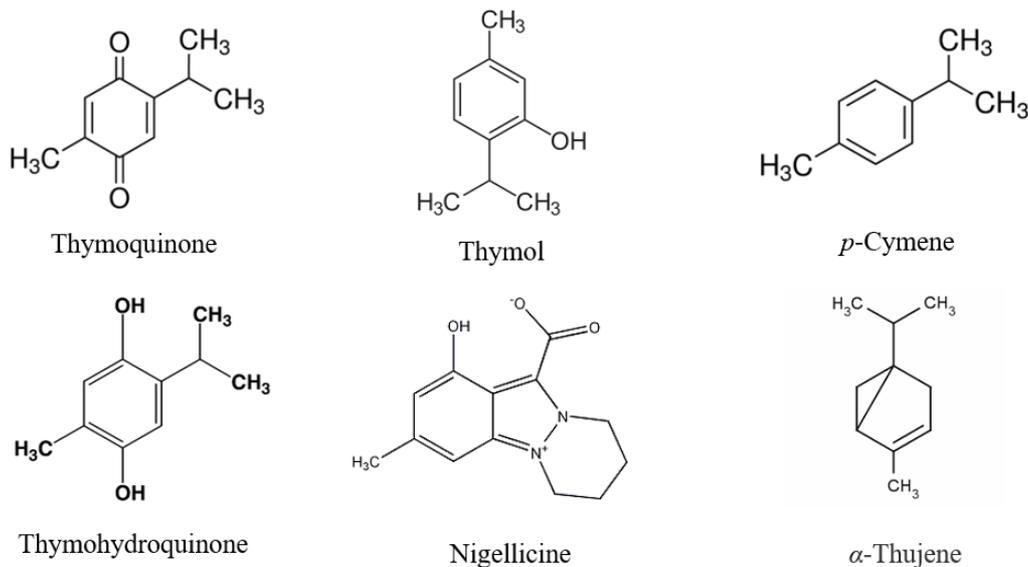


Fig. 1. Chemical structure of active ingredient of *Nigella sativa* essential oil.

2. Description and chemical composition of *Nigella sativa* essential oil

Nigella sativa seeds are reported with a rich source of many important nutrients, which contain protein, polylactide, 30-44.2% fixed oil, 0.4-1.49% essential oil, sterol, alkaloid, crude fiber, and minerals (Khan and Ather, 2006). Linoleic acid, oleic acid, palmitic acid were the main fatty acids in fixed oil. Phytochemical research of the NSEO had revealed the identification of bioactive compounds representing around 85% of the total amount (Mohamed F. Ramadan, 2016). Gas chromatography-mass spectrometer revealed the new ones: thymoquinone (42.4%), *p*-cymene (14.1%), carvacrol (10.3%), longifolene (6.1%) and 4-terpineol (5.1%) (Hossein Mahmoudvand et al., 2014b) as indicated in Table 1. A higher content of *p*-cymene (49.48%) and lower content of thymoquinone (0.79%) was found at Tunisian NSEO by GC-EIMS (Jrah Harzallah et al., 2011). High levels of *p*-cymene in NSEO may help to increase its commercial value, because this monosaccharide is important in the industry as intermediate for synthetic fragrance, drug and herbicides (Martín-Luengo et al., 2008). Very low content of thymoquinone was found in *Nigella sativa* seeds grown in Iran. Among them, the major compounds of NSEO were trans-anethole (38.3%), *p*-cymene (14.8%), limonene (4.3%), and carvone (4.0%) (Nickavar et al., 2003). Similar findings were found by

81 D'Antuono ([D'Antuono et al., 2002](#)) and they presented NSEO had higher variability of
 82 essential oil composition in relation to different sowing date. Above all, it can be
 83 inferred that the active content of NSEO from different sources is significantly different,
 84 which may be attributed to the environmental conditions, the time of sowing and the
 85 conditions of seed storage.

86

87

Table 1. Proximate composition of different source of *Nigella sativa* essential oil

N	Content of Compound								Reference
	Thymoquinone	<i>p</i> -cymene	Carvacrol	Longifolene	Terpinen-4-ol	α -Thujene	Limonene	α -Pinene	
1	4.47	56.67	1.68	0.79	0.31	16.35	2.41	3.58	(Badri et al., 2018)
2	0.79	49.48	0.55	0.30	0.38	18.93	2.89	5.44	(Jrah Harzallah et al., 2011)
3	11.27	36.20	2.12	6.32	2.37	10.03	1.76	-	(Mohamed F. Ramadan, 2016)
4	0.6	14.8	1.6	0.7	0.7	2.4	4.3	1.2	(Nickavar et al., 2003)
5	42.4	14.10	0.08	6.10	5.10	1.50	1.70	0.04	(Hossein Mahmoudvand et al., 2014b)
6	3.0	60.5	2.4	0.9	2.1	6.9	1.4	1.7	(Bourgou et al., 2010)
7	52.6	25.8	-	1.5	-	10.5	1.1	2.8	(Shaaban et al., 2015)

88

89 3. Food application of *Nigella Sativa* essential oil

90 To the best of our knowledge, some of recent studies employed essential oils in food
 91 products were collected as Table 1. Applied essential oils mechanism of action, target
 92 microorganisms and role are described.

93

94

Table 2. Essential oil applications in foods

Essential oil	Properties	Food application	Pathogens	Reference
---------------	------------	------------------	-----------	-----------

Oregano	Antibacterial activity, food preservative	Inhibitory antimicrobial effect on ready to eat vegetables (carrot & lettuce)	<i>Bacillus cereus</i> , <i>E. coli</i> , <i>Listeria</i> spp., <i>Staphylococcus aureus</i> , <i>Lactobacillus</i> spp., <i>Pseudomonas</i> spp., <i>Enterobacter</i> spp., <i>Salmonella</i>	(Gutierrez et al., 2008)
		Inactivated bacteria in iceberg lettuce	<i>Salmonella typhimurium</i>	(Gündüz et al., 2010)
		Antibacterial activity in fresh ground beef patties	<i>E. coli</i> , <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Lactobacillus plantarum</i>	(Emiroğlu et al., 2010)
		Antimicrobial activity in stored minced sheep meat	<i>S. enteritidis</i>	(A Govaris et al., 2010)
		Reduced microbial populations & extended shelf-life of fresh chicken meat & liver	Yeasts, Lactic acid bacteria, <i>Enterobacteriaceae</i> , <i>Brochothrix-thermosphacta</i> , <i>Pseudomonas</i> spp.	(Chouliara et al., 2007 ; Hasapidou and Savvaidis, 2011)
	Reduced survival rate of bacteria in Feta cheese	<i>E. coli</i> , <i>L. monocytogenes</i>	(Alexander Govaris et al., 2011)	
	Antifungal	Reduced fungal infections in tomatoes, strawberries & cucumbers	<i>Botrytis cinerea</i> , <i>Penicillium italicum</i> , <i>P. digitatum</i>	(Vitoratos et al., 2013)
Rosemary	Antibacterial	Inhibited bacteria in fresh leafy vegetables	<i>E. coli</i> , <i>L. monocytogenes</i> , <i>S. enteritidis</i>	(de Medeiros Barbosa et al., 2016)
		Reduced bacterial growth & extended shelf-life in meat products	Lactic acid bacteria	(Badia et al., 2019)
	Antifungal	Controlled fungal growth and disease in passion fruit	<i>Alternaria</i> spp., <i>Fusarium</i> spp., <i>Colletotrichum</i> spp., <i>Penicillium</i> spp.	(Waithaka et al., 2017)
	Antioxidant	Extended shelf-life of cream cheese & decreased fermentative deterioration	-	(Olmedo et al., 2013)
Thyme	Acaricides, Antimicrobial, food preservative	Toxic to red mite in poultry	<i>Dermanyssus gallinae</i>	(George et al., 2009)
		Extended shelf-life & reduced microbial population of lamb meat	<i>Enterobacteriaceae</i>	(Karabagias et al., 2011)

	Fungicidal effect	Inhibited microorganism & extended shelf-life of papayas	<i>Colletotrichum gloeosporioides</i> , <i>Rhizopus stolonifer</i>	(Bosquez-Molina et al., 2010)
		Inhibited the mycelium growth & spore germination in strawberry, tomato & cucumber	<i>Botrytis</i> , <i>Penicillium italicum</i> , <i>P. digitatum</i>	(Vitoratos et al., 2013)
Lemongrass	Antimicrobial	Inactivated microorganisms in Apple, pear, & melon juices	<i>S. enteritidis</i> , <i>E. coli</i> , <i>L. innocua</i>	(Raybaudi-Massilia et al., 2006 ;
	Antifungal, anti-aflatoxinogenic	Inhibited aflatoxin formation & fungal growth in stored rice	<i>Aspergillus flavus</i>	Paranagama et al., 2003)
Mint	Antibacterial	Inactivated bacteria in yogurt sample	<i>L. monocytogenes</i> , <i>L. innocua</i>	(Evrendilek and Balasubramaniam, 2011)
Garlic	Antibacterial, food preservative	Antibacterial effect in yogurt	<i>E. coli</i> , <i>Staphylococcus aureus</i>	(Nazari et al., 2019)
Cinnamon	Antifungal, food preservative	Extended shelf-life & reduced fungal disease incidence in bananas	<i>Colletotrichum musae</i> , <i>E. coli</i> , <i>S. Typhimurium</i> , <i>L. monocytogenes</i>	(Mehdi et al., 2010)
		Exhibited complete inhibitory effect against pathogens of pomegranate	<i>Botrytis</i> sp., <i>Penicillium</i> sp., <i>Pilidiella granati</i>	(Munhuweyi et al., 2017)
	Antibacterial	Bactericidal activity in milk	<i>L. monocytogenes</i> , <i>E. coli</i>	(Cava-Roda et al., 2012)
Clove	Antibacterial, food preservative	Inhibited bacterial growth in milk, meat & cheese	<i>L. monocytogenes</i>	(Cava-Roda et al., 2012 ; Menon and Garg, 2001)
		Inhibited bacterial growth in low-fat & full-fat soft cheese	<i>L. monocytogenes</i> , <i>S. enteritidis</i>	(Smith-Palmer et al., 2001)
Eucalyptus	Antifungal	Controlled fungal pathogens in passion fruits	<i>Alternaria</i> spp., <i>Fusarium</i> spp., <i>Colletotrichum</i> spp., <i>Penicillium</i> spp.	(Waithaka et al., 2017)
	Antiyeast activity	Inhibited development of yeast in mixed fruit juice	<i>S. cerevisiae</i> , <i>Z. bailii</i> , <i>A. pullulans</i> , <i>C. diversa</i> , <i>P. fermentans</i> , <i>P. kluyveri</i> , <i>P. anomala</i> , <i>H. polymorpha</i> .	(Kumar Tyagi et al., 2014)
Mustard	Antifungal	Inhibited fungi & yeast growth in bread	<i>Aspergillus flavus</i> , <i>Penicillium commune</i> , <i>P. roqueforti</i> , <i>Endomyces fibuliger</i>	(Nielsen and Rios, 2000)

Tea tree	Antifungal	Reduced fungal growth of cereal&barley	<i>Fusarium graminearum</i> , <i>Fusarium culmorum</i> , <i>Pyrenophora graminea</i>	(Terzi et al., 2007)
	Antimicrobial, food preservative	Inhibited microbial growth in fresh cucumber juice	<i>L. monocytogenes</i> , <i>E. coli</i> .	(Shi et al., 2018)
Nigella sativa	Antioxidant, antimicrobial, food colorant	Decresed bacterial counts in dates pastes	<i>Enterobacteriaceae</i> , <i>Staphylococcus Aureus</i> , <i>Enterococcus faecalis</i>	(Bahtiti)
	Antimicrobial	Improved physicochemical&sensory properties& extended shelf-life of domiati cheese	<i>Staphylococcus aureus</i> , <i>E.coli</i> , <i>L. monocytogenes</i> , <i>S. enteritidis</i>	(Mahgoub et al., 2013)
	Antioxidant, antifungal	Strong radical scavenging capacity&fungal growth inhibition in rape seed oil	<i>Aspergillus ssp.</i> , <i>Penicillium citrinum</i> , <i>Fusarium graminearum</i>	(Singh et al., 2005)

95

96 It is well established *Nigella sativa* has many nutritional and pharmaceutical uses.

97 Their seeds is considered to be an potential sources of edible food preservative and

98 antioxidant, and it was usually used in a variety of traditional foods such as tea, yogurt,

99 pickles, sauces, and salads, since the ancient Egyptians, Greeks, and Romans arena

100 ([Benkaci–Ali et al., 2007](#); [Hajhashemi et al., 2004](#); [Venkatachallam et al., 2010](#)). A

101 mixture of NSO with beeswax was reported to use for burns, skin infections,

102 moisturizers, joint pain reliever, or an anti-wrinkle agent ([Badri et al., 2018](#)). In addition,

103 some areas apply the oil externally, for beauty and skin diseases such as psoriasis and

104 eczema. Since long time, NSEO has been used as treatment of various diseases (e.g.,

105 asthma, bronchitis, rheumatism, headaches, and dysentery) ([Badri et al., 2018](#)).

106 Meanwhile, lots of researchers explored NSEO biological properties, such as anti-

107 inflammatory, analgesic, immunomodulatory, spasmolytic, and anti-oxidant ([Ali and](#)

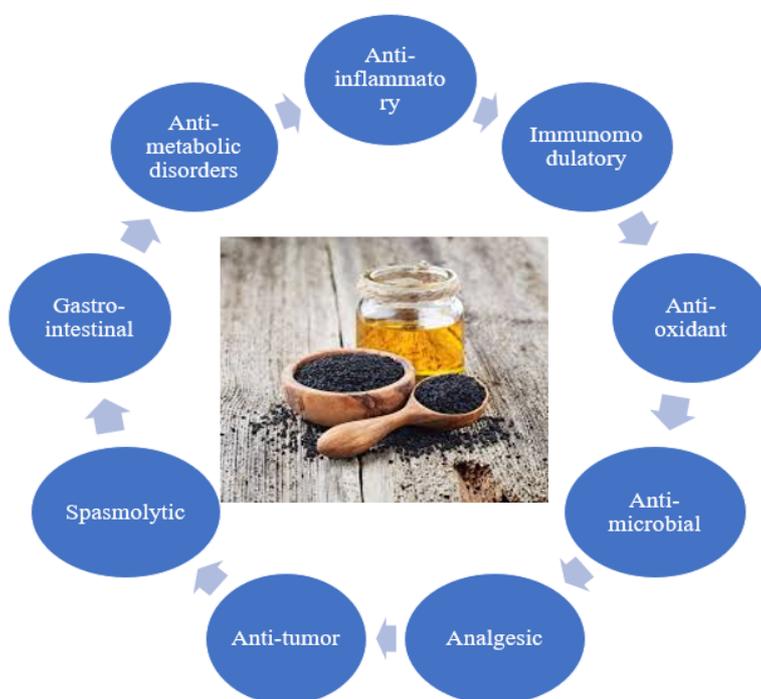
108 [Blunden, 2003](#)). Manju et al. ([Manju et al., 2016](#)) reported NSEO may be used as a

109 potential therapeutics against human lung cancer and control of pathogenic bacteria. In

110 addition, it was also shown that the NSEO has been used as a therapeutic agent to treat

111 headache, flatulence, blood hemostasis abnormalities, rheumatism, and related

112 inflammatory diseases ([Boulos and Gohary, 1983](#)). The therapeutic potential of NSEO
113 is given in Fig. 2, and these outstanding properties may use as the functional food
114 products. According to the literature, thymoquinone is major bioactive compound in
115 black cumin essential oil related to most functional properties ([Woo et al., 2012](#)).
116 Several essential oil extracted from *Nigella sativa* and their antibacterial against
117 foodborne pathogens and antioxidant activity have been studied ([Donsi et al., 2011](#);
118 [Adamu et al., 2010](#); [Sarwar and Latif, 2015](#)). Indeed, the oil extracted from *Nigella*
119 *sativa* seeds has been proved with lower toxicity ([Sultan et al., 2014](#)). So, exploring
120 their valuable uses in food industry has attracted much attention.



121

122

Fig. 2. Therapeutic impact of *Nigella sativa* essential oil on human health.

123

124 4. The advantageous properties of *Nigella sativa* essential oil for food application

125 In the food industry, avoiding food oxidation and microbial spoilage in products are the
126 most important issues. Normally, to achieve this goal, using chemical synthetic
127 additives to reduce microbial growth and delay oxidation of products are conventional
128 methods. Nowadays, as consumer awareness increases, there is a growing concern of
129 the potential carcinogenic and mutagenic risks associated with chemical synthetic

130 additives. In this sense, the development of alternative natural and low-toxic agents to
131 replace traditional synthetic substances (functional food additives) is of paramount
132 importance.

133

134 4.1. Antimicrobial Activity

135 The antibacterial activity of essential oil from plant are believed to come from phenolic
136 bioactive compounds ([Hammer et al., 2000](#)). NSEO comprising large amounts of
137 phenolic substances, such as *p*-cymene, thymol and carvacrol, which may be
138 responsible for its antimicrobial potential. Table 3 showed antibacterial activities
139 against both strains of its main constituents in NSEO and the results revealed
140 thymoquinone and longifolene had the highly active ([Bourgou et al., 2010](#)). NSEO
141 exhibited good antibacterial activity against *Staphylococcus Aureus* and *Escherichia*
142 *coli* with IC₅₀ values of 12.0 and 62.0 µg/ml ([Bourgou et al., 2010](#)). Another study
143 indicated that *Nigella sativa* active substances exhibit considerable bactericidal activity
144 and prevent the growth and development of ~90% of *Staphylococcus aureus*,
145 *Staphylococcus epidermidis* and *Enterococcus faecalis* ([Chaieb et al., 2011](#)). Sarwar
146 and Latif ([Sarwar and Latif, 2015](#)) found that NSO was more effective against
147 *Salmonella* species even than Ceftriaxone and Ciprofloxacin. Khosravi et al. ([Khosravi](#)
148 [et al., 2011](#)) evaluated the effects of NSEO against the growth and aflatoxins production
149 of *A. parasiticus* and results showed that NSEO had strong activity against *A.*
150 *parasiticus* (MIC₉₀: 2.75; MFC: 6.25 mg / mL). This study showed that NSEO can be
151 used as a natural inhibitor in low-level foods to protect food from fungal and toxin
152 contamination. Viuda-Martos et al. ([Viuda-Martos et al., 2011](#)) studied the effectiveness
153 of the Egyptian NSEO on three bacterial species (*Listeria*, *Pseudomonas*, and *Serratia*)
154 and they found it only against *L. innocua* (inhibition zones of 31.00 mm). Jrah Harzallah
155 ([Jrah Harzallah et al., 2011](#)) compared the antibacterial properties of the Tunisian NSEO
156 and its bioactive compounds (Thymoquinone). The results showed the NSEO has a
157 strong antibacterial activity against *S. mitis*, *S. mutans*, *S. constellatus* and *G.*
158 *haemolysans* (MIC=2.13 mg/ml) than only used thymoquinone, which mean the

159 different compounds in the essential oil working in a synergistic manner. This
 160 emphasizes the importance of using whole oil (or crude extract) of seeds in
 161 pharmacological research.

162

163 Table 3. Antibacterial activities of *Nigella sativa* essential oil and its main constituents. ([Bourgou et al.,](#)
 164 [2010](#))

Tested compounds	<i>S. aureus</i>	<i>E. coli</i>
<i>Nigella sativa</i> essential oil	12.0 ± 4.0 ^a	62.0 ± 17.0 ^a
<i>p</i> -Cymene	> 100 ^b	> 100 ^b
γ -Terpinene	> 100 ^b	> 100 ^b
Thymoquinone	1.8 ± 0.6 ^b	41.0 ± 19.0 ^b
β -Pinene	> 100 ^b	> 100 ^b
Carvacrol	> 100 ^b	> 100 ^b
Terpinen-4-ol	> 100 ^b	> 100 ^b
Longifolene	3.0 ± 1.0 ^b	> 100 ^b
Chloramphenicol	7.0 ± 1.0 ^b	0.8 ± 0.1 ^b

165

Chloramphenicol was used as positive control. Values are mean ± S.D..

166

167 4.2. Antioxidant Activity

168 Lipid oxidation is a crucial factor affecting food quality during food processing,
 169 marketing and storage. In the past decade, the antioxidant activity of *Nigella sativa*
 170 seeds and NSEO had been widely investigated ([Adamu et al., 2010](#)). Table 4 shown
 171 antioxidant activities of its main constituents in NSEO by DCFH-DA assay and they
 172 confirmed that the *in vivo* antioxidant activity of the essential oil is mainly due to the
 173 action of thymoquinone ([Bourgou et al., 2010](#)). It is reported that carvacrol and thymol
 174 in NSEO also contribute to antioxidant activity *in vitro* ([Houghton et al., 1995](#)). As
 175 reported by Cascella et al. ([Cascella et al., 2018](#)), *Nigella sativa* could be a useful
 176 compound for preventing and treating cerebral ischemic and neurodegenerative
 177 diseases, due to it have significant anti-oxidant effect and could represent effective
 178 neuroprotective activity. In addition, Viuda-Martos ([Viuda-Martos et al., 2011](#))

179 compared antioxidant properties of five essential oil of the species widely cultivated in
 180 Egypt. Accordingly, they found that, black cumin oil has the strongest antioxidant
 181 activity ($p < 0.05$) and its free radical scavenging was 95.89 % at 50 mg/mL. Sultan et
 182 al. ([Sultan et al., 2009](#)) studied fixed and essential oil from *Nigella sativa* and they
 183 found NSEO (80.25%) showed significant higher antioxidant capacity than fixed oil
 184 (32.32%) by the experiments of DPPH radical scavenging. Based on Erkan, Ayrançi's
 185 results, NSEO inhibited DPPH radical formation and mean the value of IC_{50} (μM) was
 186 found to be 515 ± 20.1 ([Erkan et al., 2008](#)). Another experiments about NSEO, the value
 187 of IC_{50} value of thymoquinone and carvacrol tested by DPPH assay were found to be
 188 460.0, 211.0 and 28.8 mg/mL, respectively ([Burits and Bucar, 2000](#)).

189

190 **Table 4.** Antioxidant activities of *Nigella sativa* essential oil and its main constituents. ([Bourgou et al.,](#)
 191 [2010](#))

Tested compounds	Inhibition of DCFH oxidation
<i>Nigella sativa</i> essential oil	1.0 ± 1.0^a
<i>p</i> -Cymene	$> 200^b$
γ -Terpinene	$> 200^b$
Thymoquinone	1.0 ± 0.8^b
β -Pinene	$> 200^b$
Carvacrol	190.0 ± 60.0^b
Terpinen-4-ol	$> 200^b$
Longifolene	$> 200^b$
Quercetin	0.1 ± 0.1^b

192 Quercetin was used as positive control. Values are mean \pm S.D. A= IC_{50} values ($\mu g/ml$); b= IC_{50} values
 193 (μM).
 194

195 4.3. Nutritional value

196 As an edible and medicinal plant, *Nigella sativa* is generally to take in numerous forms,
 197 such as spice, fruit, or food ingredient. In different types of meals, NSEO as a bread or
 198 cheese flavoring and spice is employed. According to the report ([Ahmad et al., 2013](#)),
 199 *Nigella sativa* seeds contain protein (26%), carbohydrates (25%), crude fiber (8.4%),

200 ash (4.8%) with large amounts of carotene and minerals (Cu, P, Zn and Fe) (Table. 5),
201 such as copper, phosphorus, zinc and iron. Tulukcu ([Tulukcu, 2011](#)) indicated that the
202 crude oil extracted from *Nigella sativa* is considered a functional oil due to its rich
203 content of omega-6 and omega-9 fatty acids including linoleic (54.0–70.0%) and oleic
204 (15.0–24.0%), respectively.

205

206 **Table 5.** Principal nutrients of *Nigella sativa*

Compound	Quantity (%)
Protein	26
Carbohydrates	25
Crude fiber	8.4
Ash	4.8

207

208 Meanwhile, there was very low toxicity of NSO have been observed in rat and mice
209 ([Zaoui et al., 2002](#)). Mahmoudv ([H. Mahmoudvand et al., 2014a](#)) evaluated the
210 cytotoxic effects of various extracts of NSEO and thymoquinone on murine
211 macrophages by MTT assay and they observed a dose-dependent response with
212 increasing concentrations. In this stage, NSEO showed a less cytotoxic effect on murine
213 macrophages and the value of C_{50} was $641.6 \pm 5.1 \mu\text{g/mL}$, while thymoquinone as the
214 active constituent showed higher cytotoxic effect ($C_{50}=36.3 \mu\text{g/mL}$). In another study,
215 Dollah et al. ([Dollah et al., 2013](#)) studied the toxicity of *Nigella sativa* powder on liver
216 function, but they did not see significant changes in liver enzyme blood levels. The
217 same result was reported by Khader ([Khader et al., 2009](#)) who extracted NSO with no
218 significant side effects on liver or kidney function. Zaoui, Cherrah et al ([Zaoui et al.,](#)
219 [2002](#)) also confirmed the low toxicity of NSO by high LD_{50} values, and suggested a
220 wide margin of safety for therapeutic doses of NSO From the above results, it can be
221 concluded that *Nigella sativa* and its extracts oils are comparatively safe as a dietary
222 supplement or a remedy for drugs, but there is still lack of systematic evaluation.

223

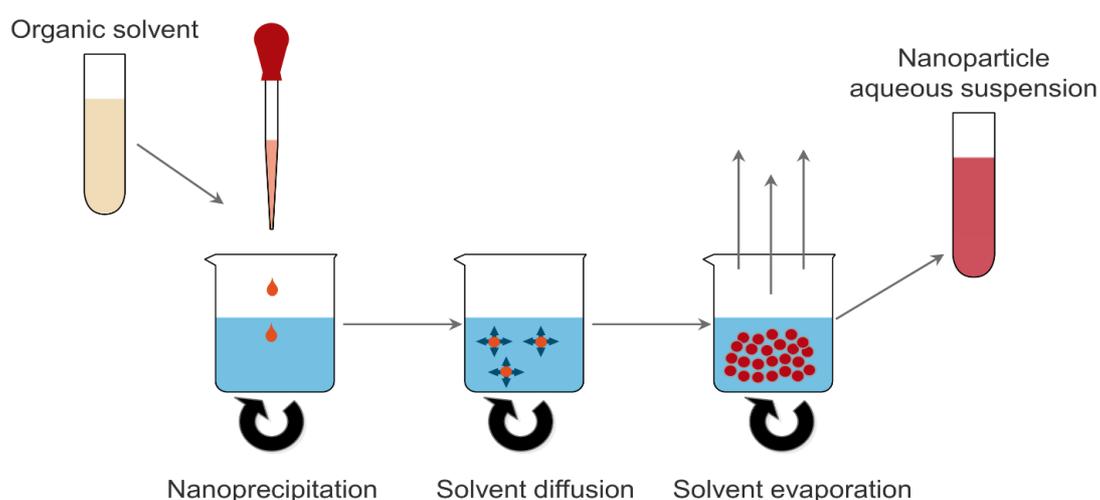
224 5. Stability of the *Nigella sativa* oil

225 Oxidation is a big problem in NSO during food processing, marketing and storage since
226 these oils are sensitive to oxidation. Oxidation not only deteriorates the taste and aroma
227 of the oils but also leads to formation of hazardous compounds. Literature has reported
228 that the progress of lipid oxidation of edible oils is influenced by many factors such as
229 light, heat treatment, oxygen availability and the presence of antioxidants
230 ([Grosshagauer et al., 2019](#)). Thus, the mechanism and prevention method of oxidation
231 must be considered. Lipid oxidation of NSO is a complex chemical reaction sequence
232 that is prone to enzymatic and non-enzymatic oxidative degradation. The happening of
233 enzymatic oxidation (hydrolysis) of oil typically requires the presence of water and
234 enzymes (such as lipoxygenases, esterase and cyclooxygenases) in the oil phase and the
235 enzymatic oxidation will lead to the production of hydro-peroxides, which may cause
236 cell damage ([Ahmed et al., 2016](#)). Non-enzymatic oxidation can be divided into auto-
237 oxidation and photo-oxidation. According to the Choe and Min's report ([Choe and Min,
238 2006](#)), auto-oxidation is mediated by autocatalytic formation and reaction of free
239 radicals (radical forms of acylglycerols) or triplet oxygen ($^3\text{O}_2$) with oils, whereas the
240 photo-oxidation is mediated by ultraviolet or singlet oxygen ($^1\text{O}_2$). The automatic
241 oxidation of NSO could be accelerated by free fatty acids, metals such as iron,
242 monoacylglycerols and diacylglycerols, and thermo-oxidic compounds. Study from
243 Mohammed, Meor Hussin et al. ([Mohammed et al., 2017a](#)), they pointed out that NSO
244 is susceptible to oxidative degradation and the peroxide value (78.57 ± 0.22 mEq O_2/kg
245 oil) of NSO showed significant increases with 24 days. Similar finding was reported by
246 Ramadan et al. ([Mohamed Fawzy Ramadan and Mörsel, 2004](#)), who reported that NSO
247 showed increasing in the peroxide value up to 64.5 mEq O_2/kg after 21 days. On the
248 other hand, NSO will oxidize degradation and NSEO will volatilize during storage.
249 Fortunately, these challenges of using NSO can be overcome to some extent by
250 suitable encapsulation technology.

251

252 6. Encapsulation of *Nigella Sativa* essential oil and their potential applications in

253 foods
254 NSO is rich in monounsaturated fatty acids (very sensitive to oxidation). A small
255 amount of essential oil (NSEO) in NSO should also be prevented from volatilization.
256 Besides that, there are some problems with low solubility and weird smell that cannot
257 be fully applied. Luckily, the incorporation of NSO or NSEO into in a shell of wall
258 material for protection can significantly improve physical properties, such as
259 dispersibility, solubility, turbidity, viscosity, and thus promote bioactivity, in
260 comparison with the free NSO ([Blanco-Padilla et al., 2014](#)).



261

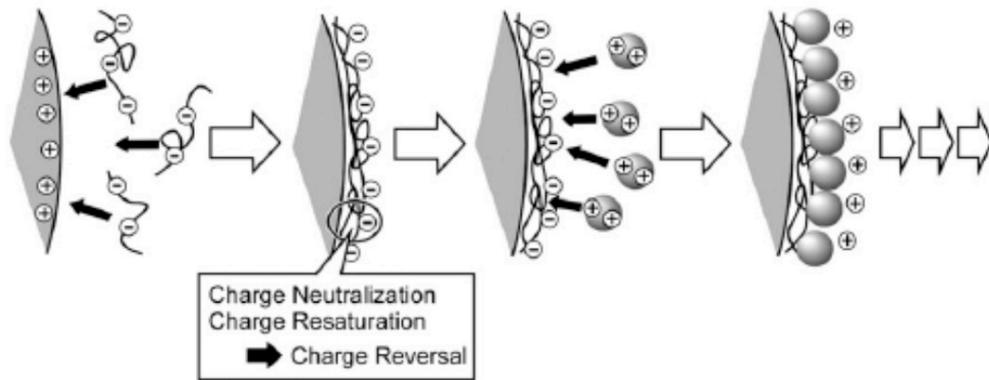
262

Fig. 3. Nanoprecipitation method ([Tarhini et al., 2017](#)).

263

264 Some encapsulation systems studied about NSO and its main founding are listed in
265 Table 5. For the food industry, nowadays, various technologies for the encapsulation of
266 bioactive compounds have been investigated; however, only a few techniques, namely
267 spray drying and freeze drying, could be used in the food industries ([de Souza Simões
268 et al., 2017](#)), because of its high efficiency, low cost and suitable equipment that is
269 readily available ([Dobry et al., 2009](#)). Meanwhile, there are also some other ways to
270 encapsulate NSO at laboratory scale, such as nanoprecipitation ([Badri et al., 2018](#)),
271 high-energy methods ([Periasamy et al., 2016](#); [Sharif et al., 2017](#)) and layer-by-layer
272 (LbL) (Fig. 4) ([Konuk Takma and Korel, 2019](#)).

273



274

275

Fig. 4 Layer by layer method (Miladi et al., 2014)

276

277 Spray drying (Fig. 5) is the most common and economically practicable method used
 278 in the manufacture of powdered form for food applications (Carneiro et al., 2013). In
 279 general, there are three main steps in the spray drying process for microencapsulation:
 280 preparation of the dispersion, homogenization of the emulsion and atomization of
 281 material into the drying chamber (Gharsallaoui et al., 2007). The wall material content,
 282 oil concentration, ratio, and spray dried inlet air temperature are the main parameters
 283 effecting the final encapsulated powder (Jafari et al., 2008). Mohammed, Tan et al.
 284 (Mohammed et al., 2017b) investigated the effect of process conditions on the
 285 microencapsulation of spray-dried NSO by the response surface methodology (RSM).
 286 Their results show that lipid oxidation of NSO could be significantly reduced by
 287 optimizing the microencapsulation conditions. The highest encapsulation efficiency
 288 reached 92.71%. Edris, Kalemba et al. (Edris et al., 2016) also used spray drying
 289 technique to encapsulate *Nigella sativa* oleoresin into the powder. The results showed
 290 that encapsulation efficiency of the volatile oil fraction reached 86.2%, which mean that
 291 it can be used in the manufacture of foods and nutraceuticals. There are some other
 292 techniques to encapsulate NSEO. Sharif, et al. (Sharif et al., 2017) used different ratios
 293 of NSEO with modified starch, under high speed homogenization, to formulate
 294 nanoemulsion. The nanoemulsion exhibited monomodal size distributions with mean
 295 droplet diameter below 200 nm and showed stability against phase separation and
 296 coalescence for 4 weeks storage at room temperature. Badri, et al (Badri et al., 2018)

297 used nanoprecipitation method (see Fig. 3) to design NSO nanocapsule by
298 nanoprecipitation. The results showed that the size of the prepared nanoparticles did not
299 change significantly within 30 days of the stability study (change interval 250-270 nm).
300 Negut, et al. (Negut et al., 2018) prepared Fe₃O₄ nanoparticles containing NSO by co-
301 precipitation method in NH₄OH solution.
302



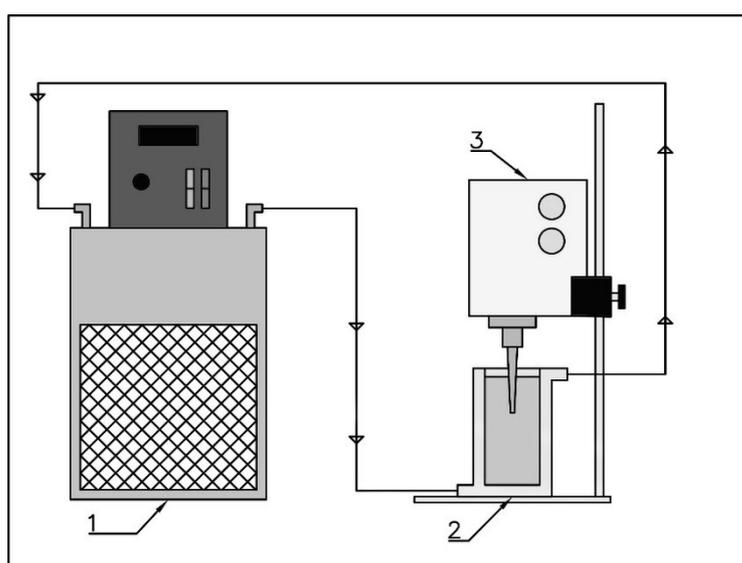
303
304

Fig. 5. The process diagram of spray drying.

305 In brief, given excellent physical properties of NSEO after encapsulation, they have the
306 potential to be used in novel food products as a food preservative, antioxidant in
307 different forms (emulsion, powder and packaging, etc). Konuk Takma and Korel
308 (Konuk Takma and Korel, 2019) prepared the film by antimicrobial chitosan and
309 alginate containing NSO and investigated the effect of NSO packaging film on the
310 quality and shelf-life of chicken breast meats stored at 4 °C for 5 days. The results
311 showed that the samples stored in the active packaging had Lower Total Aerobic
312 Mesophilic (TAMC) and Psychrotrophic Bacteria Counts (PBC) in compare with the
313 control group. Shaaban, Sadek et al. (Shaaban et al., 2015) prepared water-based
314 microemulsion system containing NSEO and its antibacterial activity against six
315 pathogenic bacteria was evaluated. Results showed that NSEO microemulsion was
316 highly effective against *S. aureus*, *B. cereus* and *S. typhimurium* even at the lowest
317 tested concentration (100.0 µg/well). Sharif, et al. (Sharif et al., 2017) indicated

318 encapsulated NSEO showed higher bactericidal activity against two Gram positive
 319 pathogenic bacterial strains as compared to pure NSEO. Abedi, Rismanchi et al. ([Abedi
 320 et al., 2016](#)) found out that NSO microcapsules can be employed to produce functional
 321 yogurt because of its high stability, proper chemical and sensory properties. Despite this
 322 fact, there are still only a few studies carried out to reveal their potential application as
 323 functional food products.

324



325

326 **Fig. 6.** Ultrasonication experimental setup. 1) cooled circulation bath, 2) double wall container, 3)
 327 ultrasonic probe ([Martínez et al., 2018](#)).

328

329 Table 6. Recently encapsulated *Nigella Sativa* oil, *Nigella Sativa* essential oil and Thymoquinone

Core material	Encapsulation techniques	Wall materials	Forms	Main conclusions	References
NSEO	Emulsification	Tween 20	Microemulsion	The EO emulsion was more effective than eugenol emulsion against <i>S. aureus</i> , <i>E. coli</i> , <i>B. cereus</i> , <i>L. monocytogens</i>	(Shaaban et al., 2015)
NSEO	High speed homogenization	Modified starch	Nano-emulsions	Emulsions showed shear thinning phenomena and stability towards coalescence during storage (4 weeks at 25 °C)	(Sharif et al., 2017)
NSEO	Layer-by-layer (LbL)	Alginate and chitosan	Film	Results indicated that active film containing NSEO has the potential to maintain safety and quality of chicken meat.	(Konuk Takma and Korel, 2019)

NSEO	Nanoprecipitation	Poly (ϵ -caprolactone)	Nanoparticles	NSEO were successfully encapsulated that would boost the anti-inflammatory and analgesic effects of indomethacin.	(Badri et al., 2018)
NSO	Spray drying	Sodium caseinate and maltodextrin DE10	Powder	The application of microencapsulation had a significant protection effect on the oil oxidation	(Tarhini et al., 2017)
NSO	Ultrasonication (Fig. 6)	Whey proteins	Emulsion	Emulsion droplets keep stable at least 8 days upon storage at 4 ± 2 °C	(Anandan et al., 2017)
NSO	Spray drying	Maltodextrin DE10 and sodium caseinate	Powder	Encapsulated NSO showed increased stability and resistance content of bioactive compounds, and antioxidant activity, as well as changes in fatty acid composition than un-encapsulated NSO	(Mohammed et al., 2017a)
NSO	Spray drying	Modified starch and maltodextrin	Powder	The results of chemical and sensory analysis suggest that NSO microcapsules can be used for producing functional yogurt	(Abedi et al., 2016)
<i>Nigella Sativa</i> oleoresin	Spray drying	Gum Arabic and Tween 80	Powder	The powder, which encapsulates a functional oleoresin, can be used in fortification of different processed foods, dairy or nutraceutical products.	(Edris et al., 2016)
Thymoquinone	Solvent evaporation method	PLGA (poly (dl-lactide-co-glycolide))	Nanoparticles	PLGA encapsulated TQ nanoparticle with sustained release property has preserved antioxidant as well as antimicrobial activity	(Nallamuthu et al., 2013)
Thymoquinone	High-pressure homogenization	Sorbitol, Tween 80 and thimerosal	Nanostructured lipid carriers (NLC)	NLCs could be a promising vehicle for the oral delivery of TQ and improve its gastroprotective properties	(Abdelwahab et al., 2013)

330

331 7. Conclusion

332 Interest in new sources of oil seeds has recently grown. This chapter conclude that
333 among the various oil seeds, NSEO containing several phytochemicals that has been
334 considered all over the world for having a positive impact on human health. Particularly,
335 essential oil in *Nigella sativa* displays considerable commercial value thanks to its
336 strong antimicrobial and antioxidant activities. These outstanding properties of NSEO
337 have potential to play a remarkable role as a novel source of food preservative or

338 antioxidants. However, there are still some physical shortcomings like lipid oxidation,
339 low water solubility and volatility. The recent advancement in encapsulation, offers the
340 great opportunity to design novel carriers to deliver NSEO and to control its release.
341 Therefore, further research is needed to be conducted to find new applications of
342 *Nigella sativa* and to transfer NSEO encapsulation from laboratory-scale to industrial
343 scale.

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