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Chemical Investigation and Preservative Effect of Jordanian *Nigella Sativa* L. Seed Oil on Date Paste

Nawal.H.Al.Bahtiti

Applied Science University (Private)
Amman-Jordan

Abstract: *One of the miracle herbs plant that has been considered as prophetic medicine is Habbatus sauda (Nigella Sativa).Nigella sativa also known as Black seed is an annual herbaceous plant and it is indigenous to the Mediterranean region.HPLC showed that Jordanian Nigella Sativa contain a variety of pharmacologically active substances like thymoquinone, dithymoquinone, thymol, carvacrol, nigellicine-N-oxide, nigellidine and alpha-hederin. The effects of Nigella sativa oil (100-400ppm) on poisoning and pathogenic bacteria were examined. Nigella sativa L and its various extracts were found to possess antibacterial activity against gram positive and gram negative bacteria, and they caused inhibition of Staphylococcus aureus, Enterobacteriaceae, and Enterococcus faecalis .Nigella sativa L., was tested for a possible antioxidant activity, showed that thymoquinone demonstrated respectable radical scavenging property. In this study, the possibility of natural antimicrobial compounds, Jordanian Nigella sativa L as food preservatives is investigated. The experiment was conducted in the laboratories of basic science, at applied science university to optimize the preservative for safe storage of date pastes. The post-processing development of contaminating microorganisms present in stored date pastes was controlled effectively with 100, 200, and 400ppm of Jordanian Nigella sativa. Among these preservatives, 400ppm Nigella sativa showed better sensory quality attributes in respect of color, flavor, texture and taste during four months of storage at room temperature, which is better than 400 ppm of sodium benzoate preservatives. Antimicrobial effect was tested during storage at 25˚C. When the microorganisms were treated with Nigella sativa L, the population of food spoilage microorganisms decreased by 2.6-3.7 log cycles. The inhibitory effect was increased in more acidic condition. Nigella sativa was effective at preventing microbial growth and extending shelf-life,So black seeds are recommended to be used in food like flavoring additive in the breads and pickles because it has very low level of toxicity.*

Keywords: *Nigella sativa, black seed, thymoquinone, pathogenic bacteria, antioxidant activity*

1. INTRODUCTION

Nigella sativa is an annual flowering plant in the family Ranunculaceae, native to south and southwest Asia, In the ethnopharmacology of those countries, N. sativa seeds are used to cure gastro-intestinal disorders as well as skin or respiratory ailments [1]. Several other pharmacological properties have been traditionally attributed to N. sativa seeds, simply as a crushed powder, or as an extract. Purified or as a mixture, metabolites of N. sativa seeds would present a potent and therapeutically interesting activity on the cardiovascular, respiratory, immune, and endocrine systems [2-4]. Additional properties are frequently discovered [5]. Most of these activities have already been attributed to thymoquinone, a major component of the essential oil of the seeds [6]. Additionally, in its native range and far beyond, N. sativa seeds are also frequently used as spice and condiment in various recipes due to their characteristic aroma and bitter and peppery taste [7]. Finally, N. sativa seeds are used to prepare highly prized nutritive oil. Although on the world scale Nigella seed oil does not really have a significant economic market share, yet, it nevertheless constitutes a niche market whose size is constantly growing due to its alleged pharmacological Plant extracts and essential oil showed a broad range of pharmacological effects such as anti diabetic [8]. The extracts of the plant also showed anti microbial effects [9].Seeds of Nigella sativa L. (Ranunculaceae) commonly known as black seed or black cumin, are used in folk (herbal) medicine all over the world for the treatment and prevention of a number of diseases and conditions that include asthma, diarrhea and dyslipidaemia [6]. Seeds of Nigella sativa have been employed for thousands of years as a spice and food preservative; one of the potential properties of Nigella sativa seed is the ability of one or

more of its constituents to reduce toxicity due its anti-oxidant activities. Therefore the use of Nigella sativa in food is recommended to suppress lipid oxidation and may be useful as natural food preservative. And an alternative to synthetic preservatives that are harmful to health. Thus, the objective of this study was carried out to extract of oil from N.sativa and to evaluate the preservative/antioxidant activity of oils in dates pastes after accelerated oxidation at 65 ° C .Moreover, the effects of different concentrations of Nigella sativa combined pastes of dates samples were studied, microbiological, chemical and sensory characteristics of samples during room temperature storage 25° C± 1 of dates pastes were investigated.

2. MATERIALS AND METHODS

Fresh seeds of Nigella Sativa were obtained from Almushaqer region at Jordan, were cleaned and ground to fine powder. Fresh Jordanian date was purchased from local market. Removing pits and converted into paste.

Preparation of date paste samples blended with Nigella sativa oils, Samples of date paste blended with the best ratio 400ppm of Nigella sativa oil. The samples were packaged in tightly sealed polyethylene pouches (0.2 ± 0.10g) and divided into four groups, the first used was control, second were treated samples at ratio of 100ppm Nigella sativa, third samples at 200ppm, and fourth treated samples with 400ppm of Nigella sativa. Then all samples were stored at room temperature (25± 1°C) for sensory evaluation.

2.1. Sensory Evaluation

Dates pastes samples were periodically examined (every 7 days) for their appearance, texture and odor post treatments and during room temperature storage at 25 ± 1 □C to determine the shelf-life of the samples. The panel consisted of ten members from our laboratory and scores were obtained as described by Wierbicki [10] by rating the above quality characteristics using the following rating scale:9 excellent,8=very good,7=good,6=below good above fair,5=fair,4=below fair above poor,3=poor,2=very poor and 1=extremely poor.

2.2. Microbiological Analysis

Colony forming units for total bacterial count were counted by plating on plate count agar medium and incubation at 30°Cfor 3-5 days [11]

Enterobacteriaceae were counted on violet red bile glucose agar medium after incubation for 20-24 h at 37°C [12].

Staphylococcus aureus was counted using Baird–Parker medium after incubated at 35°C for 24-48 h [13-14].

Enterococcus faecalis was counted on kanamycin aesculine azide agar medium using surface plating technique and incubation at 35°C for 16-24 h according to the Oxoid manual (1998).

3. RESULTS& DISCUSSION

As shown in Table 1, specific gravities estimated with N. sativa (0.9071 at 25 ^{o}C) and seed oils were higher than the value 0.8840 at 30 ^{o}C for seed oil reported by Mariod et al. [15]. Refractive indices of the oils were found to be 1.4683 for N. sativa at 30^{o}C, being higher than 1.4340 at 40 ^{o}C for the Cucumis sativus seed oil [15]. Iodine values estimated for N. sativus were 114. Iodine value of N. sativa was lower than 119 reported by Salma et al. [16] for same seed oil of Tunisian variety. The iodine values obtained in this study indicate that the N. sativa seed oils contain high level of unsaturated bonds. Therefore, the samples in the present investigation have higher tendency to become rancid by oxidation. The comparatively the saponification value of the sample seed oil (203) as estimated indicates the presence of higher proportion of higher fatty acids. The investigated saponification value for N. sativus was lower than the values 211-218 mentioned by Salma et al. [16], but similar to 203 mentioned by Atta [17] for the same seed oil. The FFA content (12%) in N. sativus seed oil was similar to the value 11 cited in the literature [1], but much higher than 1.3 for Cucumis sativus [15]. Results regarding FFA contents indicate the suitability of the oil sample of Jordanian Nigella sativa for preservative purpose as it contained significantly lower percentage of FFA than that contained in the sample of N. sativus. The high acidity of oil may be related to the nature of N. sativus seed. The oxidative stabilities of

sample with no antioxidant (control) is less than the samples with Nigella Sativa oil or seed, sample with sodium benzoate is less than stability with samples with Nigella Sativa. Stability decreased by times.

Table1. *Physical and chemical characteristics of Nigella sativa oil*

Characteristics	
Specific gravity at 25 °C	0.9071± 0.0041
Refractive index at 28 °C	1.4683 ± 0.0030
Iodine value (g of I2/100 g of oil)	114 ± 0.8
Saponification value (mg KOH/g)	203 ± 2
Free fatty acids (%) as oleic	12 ± 0.1
Unsaponifiable matter (g/100 g)	1.2 ± 0.7
Peroxide value (mEq/kg of oil)	12.7 ± 0.1
Reichert-Meissl value	0.97 ± 0.1
Acetyl value	3.5 ± 0.2

Table 2 and figure 1 shows high percentage of thymoquinone 52.5 % constituent of N.sativa.

Table2. *Percentage composition of N. sativa*

Compounds	Composition (%)
Nigellicine-N-oxide	1.7 ± 0.1
Nigellidine	16 ± 0.2
Alpha-hederin	4 ± 0.3
dithymoquinone	22.4 ± 0.5
thymoquinone	52.5± 0.7
Carvacrol	1.3 ± 0.1
thymol	2.1 ± 0.1

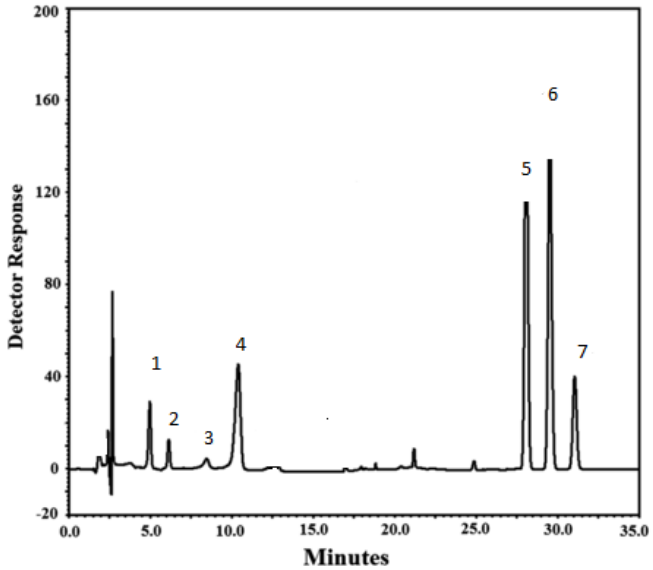

Figure1. *HPLC for Nigella sativa oil (thymoquinone (5), dithymoquinone, (4), thymol (7), carvacrol (6), nigellicine-N-oxide (1), nigellidine (2) and alpha-hederin. (3).*

Table 3, 4 shows high nutrition value of Nigella sativa oil which is added to date pastes.

Table3. *Nutrient contents of N. sativa.*

Parameters (g/100 g dry weight basis)	
Moisture	5.2 ± 0.3
Lipid	36 ± 0.4
Ash	4 ± 0.3
Total protein	18.8 ± 0.3
Water soluble protein	5.5 ± 0.3
Starch	4.0 ± 0.3
Crude fiber	5.2 ± 0.3
Total sugar	1 ± 0.2
Total carbohydrate	30

Table4. *Mineral contents of N. sativa*

Parameters (mg/100 g dry weight basis)	
Calcium	611 ± 3
Copper	4.8 ± 0.4 5.4 ± 0.3
Iron	11.2 ± 0.3
Zinc	6.2 ± 0.3
Potassium	802 ± 2.3
Magnesium	80.2 ± 0.2
Phosphorus	118 ± 2.2
Sodium	280 ± 1
Manganese	1.4 ± 0.2

Table 5 shows the results of sensory evaluation of N.sativa. A sample with 400ppm has a good color, favor, and appearance, better than 400ppm sodium benzoate. The common causes for spoilage of date pastes are microbiological deterioration and lipid oxidation; whereas the major limiting factor for products is lipid oxidation, which may affect acceptability of food because of rancidity, and it may decrease the nutritional value by forming potential toxic products during processing [18].

Table5. *Sensory evaluation of mixtures of dates pastes and Nigella Sativa L after 21 days*

Blending ratios (w/w)	Color	Flavor	Appearance
Control	4	5	4
100ppm NS oil	6	7	6
200ppm NS oil	8	8	8
400ppm NS oil	9	9	9
400ppm NS seed	8	7	7
400ppm sodium benzoate	7	6	6

The data in Table 6 exhibits that the effects of combination treatments between Nigella seeds and the ratios of oil extracted from Nigella seeds powder on the microbial load in date pastes fillets samples during room storage at 25 ±1°C.

The high level for initial bacterial counts may be due the possible contamination during handling

Was 8.6×10^6, presence of N.sativa decresed the bacterial counts.

Table6. *Effects of combination treatments of Nigella sativa seed, and sodium benzoate on date pastes.*

Microbial Determinations (fcu/g)	Storage Periods days	Control	100ppm cumin seed	100ppm N.S oil	200ppm N.S oil	400ppmN .S oil	400ppm sodium benzoate
Enterobacteriaceae	0	6.0×10^3	3.4×10^3	2.5×10^3	6.7×10^2	3.4×10	7.4×10^2
	7	9×10^3	7.8×10^3	5.5×10^2	8.5×10^2	5.1×10	9.3×10^2
	14	11.7×10^3	4.7×10^4	7.6×10^2	2.6×10^3	8.4×10	6.2×10^3
	21	2.1×10^4	9.5×10^4	9.8×10^3	7.3×10^3	9.7×10	4.3×10^4
Staphylococcus aureus	0	3.9×10^4	1.2×10^3	6.7×10^2	4.1×10^2	5.3×10	7.8×10^2
	7	7.2×10^4	7.6×10^3	8.3×10^2	6.3×10^2	6.7×10	9.3×10^2
	14	9.2×10^4	8.2×10^3	9.6×10^2	7.6×10^2	5.9×10^2	9.8×10^2
	21	2.3×10^3	1.2×10^4	4.7×10^3	3.2×10^3	7.8×10^3	8.7×10^3
Enterococcus faecalis	0	6.8×10^4	6.5×10^3	1.1×10^3	2.5×10^2	1.2×10^2	2.4×10^2
	7	9.6×10^4	9.8×10^3	3.7×10^3	4.5×10^2	2.6×10^2	3.7×10^2
	14	5.6×10^5	3.6×10^5	4.8×10^4	3.1×10^3	6.6×10^2	8.5×10^2
	21	8.7×10^6	7.5×10^5	6.7×10^4	6.8×10^3	9.8×10^2	3.8×10^3

4. CONCLUSION

Generally, it can be concluded that the oil extracted from Nigella sativa seeds can be used as good natural preservative (antioxidant and antimicrobial) and source to develop desirable properties for samples under investigation at room temperature (25 ± 1°C).

Thus, this study recommended that Nigella Sativa is useful as natural food colorant and preservative in many products and an alternative to synthetic dyes that are harmful to health.

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