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# Effect of *Nigella sativa* L. essential oil on oxidative stability and microbial growth of local white Feta cheese

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**Abstract**

Controlling the development of pathogenic and spoilage microorganisms is critical for food manufacturers to preserve their products. Cheese provides favourable conditions for microbial growth and oxidative degradation due to the presence of water, fat, protein, and minerals. The present work thus aimed to determine the influence of *Nigella sativa* L. essential oil on microbial growth and oxidative stability of cheese made from fresh milk. The analysis of the chemical composition of *N. sativa* essential oil was performed by gas chromatography-mass spectrometry. The main compounds detected were 9,12-octadecadienoic acid (27.96%), *cis*-vaccenic acid (20.69%), 6-epi-shyobunol (7.08%), benzene, 1-methyl-3-(1-methylethyl)- (6.33%), *n*-hexadecanoic acid (3.86%), *o*-cymene (2.77%), and *cis*-4-methoxy thujane (2.71%). The antioxidant activity of *N. sativa* essential oil was calculated by the 2,2-diphenyl-1-picrylhydrazyl method with $IC_{50}$ = 8.08 µg/mL. The microdilution test showed that the minimum inhibitory concentration (MIC) of *N. sativa* essential oil for all pathogens was 500 µg/mL, while the minimum bactericidal concentration (MBC) was 1,000 µg/mL for *Staphylococcus aureus* and *Bacillus cereus*, and 2,000 µg/mL for *Escherichia coli* and *Pseudomonas aeruginosa*. The physicochemical properties of the cheese showed that the addition of essential oil did not affect the dry matter, but increased the pH, decreased the % acidity, and decreased the peroxide value compared to the control sample (sample without essential oil). In addition, the essential oil reduced the bacterial and fungal counts of the cheese compared to the control sample, and affected the sensory characteristics of the cheese during the 90-day storage period. Results also showed that the addition of 0.5% essential oil of *N. sativa* not only prevented microbial growth and oxidative degradation, but also improved the taste and overall acceptability of cheese.

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## Introduction

Cheese is one of the most famous and useful dairy products produced all over the world. The history of cheese-making goes back centuries ago. The method of cheese making was probably discovered by accident, but later it was proven to be a good way to preserve milk, which spoils quickly. Cheese is a dynamic biochemical product, and undergoes significant changes during its maturation. The flavour of cheese, including volatile and non-volatile components, is an important parameter for customer satisfaction, quality, and variety of the product (McSweeney and Sousa, 2000; Hayaloglu and Karabulut, 2013). Proteolysis and lipolysis are the main biochemical processes affecting the flavour characteristics of cheese. Proteolysis causes hydrolysis of casein into various lower molecular weight peptides and free amino acids. Subsequently, the free amino acids are converted by catabolism into amine, aldehyde, phenol, indole, alcohol, and ammonia (McSweeney and Sousa, 2000).

Studies have shown that the addition of spices such as black seed can affect the proteolysis, volatile compounds, and sensory properties of cheese (Hayaloglu and Kirbag, 2007; Hayaloglu and Karabulut, 2013; Çakır and Çakmakçı, 2018). Black seed (*Nigella sativa* L.) is one of the most valuable herbal medicines and a spice species of family Ranunculaceae that has been used for more than 2,000 years (Salem, 2005). This plant has beneficial effects such as antibacterial, antifungal, antioxidant, anti-inflammatory, digestive, antidiabetic, anticancer, hepatoprotective, antihypertensive, and immune-

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strengthening properties (Ramadan, 2007). Moreover, this herbal medicine increases appetite and lactation, and has therapeutic properties in conditions such as cataracts, lack of breast milk, asthma, infections, flatulence, rheumatism, headache, and anorexia (Ramadan, 2007). Black seed has attracted much attention due to its content of important minerals, fatty acids, and vitamins. Since the black seed is nutritious, tasty, and suitable for ornamentation, it is used all over the world (Ramadan, 2007; Çakmakçı and Çakır, 2011; Hayaloglu and Karabulut, 2013; Cakir *et al*., 2016).

It should be noted that cheese made from raw milk is one of the foods sensitive to microbial activity and oxidative stress, which can be preserved by accelerating the lactic acid fermentation process to quickly increase the acidity, or by adding chemical preservatives and maintaining hygienic conditions during production and handling. Based on the many beneficial effects of *N. sativa*, its essential oil could be used as a natural additive in cheese making (Cakmakci, 2011; Çakır and Çakmakçı, 2018). The effects of the addition of *N. sativa* essential oil to Tulum cheese on its sensory properties and volatile profiles have been published before (Cakir *et al*., 2016). Therefore, the present work aimed to investigate the effect of *N. sativa* essential oil with antimicrobial and antioxidant properties on the microbial growth and oxidative stability of cheese made from raw milk.

## Materials and methods

*Preparation of N. sativa essential oil*

Thirty grams of ground *N. sativa* was poured into a 250 mL Erlenmeyer flask with a lid with 100 mL of the solvent hexane (Merck Company, Germany). Then, it was placed in a shaking incubator at 40 rpm for 4 h. After this time, the mixture was filtered twice with Whatman paper No. 1 (Merck Company, Germany), and the extract was placed in the rotary flask under vacuum until complete evaporation of the solvent (Dalli *et al*., 2021).

*Identification of N. sativa essential oil chemical compositions*

The essential oil compounds were identified by gas chromatography and mass spectrometry (GC-MS), and obtaining their mass spectrum. A chromatography instrument (Scion-456-SQ-Netherlands) with 25 m capillary column, an inner diameter of 0.25 mm, and an inner layer thickness of 0.25 μm was used, with a column temperature program initially set at 45°C, with a pause of 2 min, then the temperature was increased to 220°C at a rate of 3°C per minute for 5 min, stopped, and the column temperature was increased to 270°C at a rate of 15°C per min for 5 min. The injection volume was 1 μL. Helium gas in split mode was used as the carrier gas, the temperature of the injection chamber was 250°C, and the flow rate of helium gas was 1 mL/min. The split ratio was set to a ratio of 1 to 100, the ionisation energy was 70 eV, and the temperature of the ionisation source was 230°C. The components of *N. sativa* essential oil were identified with the help of a mass database from the National Institute of Standards and Technology (NIST) (Kabouche *et al*., 2009; Dalli *et al*., 2021; Moradi *et al*., 2023).

*Determination of antibacterial properties of N. sativa essential oil*

The antibacterial activity of *N. sativa* essential oil against *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Bacillus cereus* (ATCC 11778) was determined by the microdilution method. The bacterial strains were provided by the Iranian Research Organization for Science and Technology (IROST, Iran). To activate the bacteria, they were first transferred to Mueller Hinton Broth (MHB) (Merck Company, Germany), and kept in an incubator at 37°C for 18 h. Then, the bacterial suspension was added to the tube containing Ringer's solution until a turbidity of 0.5 McFarland ($1.5 \times 10^8$ CFU/mL) was achieved. To determine the minimum inhibitory concentration (MIC) of *N. sativa* essential oil on the growth of pathogenic bacteria, the two-fold dilution method was used. First, the essential oil with a concentration of 16,000 μg per 1 mL of dimethyl sulfoxide (DMSO) was prepared as a stock solution, and sterilised by filtration through a 0.2-μm filter (Millipore, Billerica, MA, USA). For this purpose, 100 μL of MHB was first added to each well of the 96-well microplates. Then, 100 μL of the stock solutions were added to the first wells, and two-fold dilutions were performed in each row with concentrations of 8,000, 4,000, 2,000, 1,000, 500, and 250 μg/mL. Then, 2 μL of the bacterial suspension was added to all wells in each row to increase the final concentration of bacteria to approximately $10^6$ (CFU/mL). Finally, the microplate was kept in an incubator at 37°C for 24 h. The lowest essential oil

concentration that did not cause turbidity due to bacterial growth in the well was determined as the MIC of *N. sativa* essential oil. The microwells without visible bacterial growth were cultured on Mueller-Hinton agar (MHA) (Merck, Darmstadt, Germany). Minimum bactericidal concentration (MBC) was defined as the lowest concentration of *N. sativa* essential oil that suppresses colony formation after 24 h of incubation at 37°C (Ferraro, 2000; Moradi *et al*., 2023). Sterile MHA served as a negative control.

*Determination of antioxidant properties of N. sativa essential oil*

The effect of *N. sativa* essential oil on diphenylpicrylhydrazine (DPPH) radical scavenging was investigated. For this purpose, 25 µL of different dilutions (20, 40, 1, 10, 0.1, and 0.01 µg/mL) of *N. sativa* essential oil were first added to 2.5 mL of a 0.004% methanol solution of DPPH. The resulting mixture was shaken for 10 min, and then kept in a dark place at room temperature for 15 min. Then, the absorbance at 517 nm was read, and the percentage of inhibition was determined using Eq. 1. The $IC_{50}$ (µg/mL) is the antioxidant concentration that scavenges 50% of DPPH free radicals (Cuendet *et al*., 1997; Moradi *et al*., 2023).

$$\text{Inhibition percent} = \frac{AC - AS}{AC} \times 100 \qquad \text{(Eq. 1)}$$

where, AC = absorbance of the control, and AS = absorbance of the sample.

*Cheese-making*

In the first step, fresh cow's milk was heated to 72°C for 15 s, and then brought to a temperature of 35°C. Next, 10 L of the milk was poured into each of the sterile cheese-making containers. Then, 1/2 teaspoon of mesophilic starter CHN-22 culture (CHR Hansen, Denmark) was added and placed in an incubator until the pH reached about 5.4. After reaching the appropriate pH, 0.15 ml/L of rennet was added to the milk, and at the same time, the essential oil of *N. sativa* was added separately at three concentrations (0, 0.5, and 1%), and stored at 30°C. After 1 h, the formed curd was cut into 1 - 2 cubic cm pieces, and put under the pressure of a 5 kg weight for 4 h to extract water. Then the dehydrated curd was formed and transferred to 12% saline water (w/v), and stored at 10°C.

*Physicochemical properties of cheese*

The physicochemical properties of the cheese, including % dry matter, % fat content, peroxide, % acidity, and pH were determined following the methods of Iran National Standards Organization ISIRI 1753, 760, 19197, 2852, and 2852, respectively.

*Microbiological analysis*

The total bacterial count and fungal count were determined following the methods of Iran National Standards Organization ISIRI 5272-1 and 10154, respectively.

*Sensory evaluation*

After an introductory training on sensory evaluation, ten evaluators were selected. A 5-point hedonic test was used to indicate the evaluation of cheese samples in terms of colour, texture, taste, and overall acceptability, from very unfavourable (1) to very favourable (5).

*Statistical analysis*

The effects of storage time at four levels (1, 30, 60, and 90 days) and essential oil concentrations at three levels (0, 0.5, and 1%) on microbial growth and oxidative stability of cheese were examined with ANOVA ($p < 0.05$) using SPSS, version 16.

## Results and discussion

The present work attempted to determine the most suitable concentration of *N. sativa* essential oil to prolong the shelf life of cheese while maintaining its organoleptic properties, and for this purpose, we studied the physicochemical properties and microbial flora of cheese at different time intervals and with different concentrations of *N. sativa* essential oil.

*Analysis of chemical composition of N. sativa essential oil by GC-MS*

The results of GC-MS analysis of the compounds in the essential oil of *N. sativa* are shown in Table 1. A total of 31 compounds were identified, accounting for 94.16% of the total components. Among them, the most important compounds were 27.96% 9,12-octadecadienoic acid (linoleic acid); 20.69% *cis*-vaccenic acid ((11Z)-11-octadecenoic acid); 7.08% 6-epi-shyobunol; 6.33% benzene, 1-methyl-3-(1-methylethyl) (β-cymene, m-cymene);

**Table 1.** Chemical compositions of *Nigella sativa* essential oil.

| Chemical composition | Retention time | % of total |
|---|---|---|
| 9,12-Octadecadienoic acid (Z,Z)- | 54.303 | 27.968 |
| *cis*-Vaccenic acid | 54.51 | 20.694 |
| 6-epi-Shyobunol | 52.894 | 7.087 |
| Benzene, 1-methyl-3-(1-methylethyl)- | 11.318 | 6.338 |
| *n*-Hexadecanoic acid | 49.253 | 3.864 |
| *o*-Cymene | 10.832 | 2.773 |
| *cis*-4-Methoxy thujane | 16.155 | 2.715 |
| Phenol, 2-methyl-5-(1-methylethyl)- | 24.556 | 2.23 |
| Thymoquinone | 21.323 | 1.679 |
| Phenol, 4-methoxy-2,3,6-trimethyl- | 34.694 | 1.675 |
| 13-Docosenamide, (Z)- | 69.811 | 1.499 |
| Methyl 9-*cis*,11-*trans*-octadecadienoate | 52.736 | 1.496 |
| Octadecanoic acid | 55.141 | 1.385 |
| Longifolene | 29.008 | 1.268 |
| 1,3,3-Trimethoxybutane | 4.062 | 1.187 |
| Caryophyllene oxide | 41.053 | 1.019 |
| Retinoic acid | 53.453 | 0.967 |
| Phenol, 2,4-bis(1,1-dimethylethyl)- | 33.36 | 0.807 |
| *cis*-4-methoxy thujane | 15.106 | 0.653 |
| C(14a)-Homo-27-norgammacer-14-ene | 75.984 | 0.653 |
| Terpineol (3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-) | 18.63 | 0.604 |
| 3-Cyclohexene-1-carboxaldehyde, 1,3,4-trimethyl- | 19.845 | 0.506 |
| *o*-Cymene | 10.725 | 0.499 |
| *p*-Cymen-2-ol (Phenol, 2-methyl-5-(1-methylethyl)-) | 23.607 | 0.489 |
| 9,12-Octadecadienoic acid (Z,Z)- | 59.511 | 0.456 |
| 13-Docosenamide, (Z)- | 69.994 | 0.453 |
| Hexadecanoic acid, methyl ester | 47.859 | 0.441 |
| 2,2-Dimethoxybutane | 2.715 | 0.435 |
| Carvacrol (Phenol, 2-methyl-5-(1-methylethyl)-) | 23.447 | 0.423 |
| *cis*-4-Methoxy thujane | 15.583 | 0.359 |
| 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester | 67.618 | 0.331 |
| Phenol, 4-methoxy-2,3,6-trimethyl- | 34.234 | 0.324 |
| Carvacrol (phenol, 2-methyl-5-(1-methylethyl)-) | 23.941 | 0.298 |
| Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester | 63.516 | 0.297 |
| Thymol (*p*-Cymene, 3-hydroxy-) | 23.77 | 0.296 |

3.86% *n*-hexadecanoic acid; 2.77% *o*-Cymene; and 2.71% *cis*-4-methoxy thujane.

Identification and analysis of the essential oil components are necessary to understand their functions. In a study conducted by Hosseinzadeh and Parvardeh (2004), the major constituents of *N. sativa* essential oil were identified as thymoquinone, *ρ*-cymene, carvacrol, *t*-anethole, 4-terpineol, and longifolene. The differences in the essential oil components of a plant species may be due to differences in the geographical areas from which the essential oil plant source is grown, soil changes, weather and climate changes, plant maturity stages, harvest times, parts of the plant used to prepare the essential oil, methods of extracting the essential oil, and types of solvent used (Moosavi-Nasab *et al*., 2016).

*Antioxidant effects of N. sativa essential oil*

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is considered a simple and rapid method for determining radical scavenging activity. The results showed a considerable ability to inhibit DPPH radicals by *N. sativa* essential oil, $IC_{50}$ = 8.08 µg/mL. In previous studies, different $IC_{50}$ values of *N. sativa* were reported, 4,000.02 µg /mL in oil (Albakry *et al*., 2022), 17 µg/g (Zouirech *et al*., 2022), and 55.2 ± 2.1 µg/mL in essential oil (Harzallah *et al*., 2011). Solati *et al*. (2014) also reported that *N. sativa* essential oil contained antioxidant properties. The antioxidant activity of *N. sativa* can be attributed to its antioxidant constituents such as 4-terpineol, carvacrol, thymoquinone, and *t*-anethole (Burits and Bucar, 2000). Differences in the antioxidant activity of seed oils can be attributed to the following factors including phenolic compounds of plants; these chemical compositions are dependent on geographic locations, seed genetics, and postharvest operations (Vaya *et al*., 1997; Cowan, 1999).

*Evaluation of antibacterial properties of N. sativa essential oil*

The results of the antibacterial properties of *N. sativa* essential oil evaluated by the microdilution method are shown in Table 2 which shows *S. aureus* (MIC: 500 µg/mL; MBC: 1,000 µg/mL) and *B. cereus* (MIC: 500 µg/mL; MBC: 1,000 µg/mL) to be more sensitive than *P. aeruginosa* (MIC: 500 µg/mL; MBC: 2,000 µg/mL) and *E. coli* (MIC: 500 µg/mL; MBC: 2,000 µg/mL). Hassanien *et al*. (2015) concluded in a study that the essential oil of *N. sativa* has a growth inhibitory effect against both Gram-negative and Gram-positive bacteria, including *P. aeruginosa*, *Bacillus subtilis*, *B. cereus*, and *S. aureus*. In one study, the effect of *N. sativa* essential oil from five different geographical areas on resistant bacteria was investigated. They found that *N. sativa* essential oil can potentially be used for preventive and therapeutic purposes against multidrug-resistant bacteria, including *S. aureus*, *E. coli*, and *P. aeruginosa* (Dalli *et al*., 2021). One of the most important mechanisms proposed to explain the antibacterial effect of essential oils is that these compounds would penetrate the bacterial membrane due to their hydrophobic properties, and by disrupting the cell structure, it causes ions and cell contents to escape and damage the bacteria. The reason why Gram-negative bacteria are more resistant to essential oils than Gram-positive bacteria is the presence of an outer layer on the wall of Gram-negative bacteria, which partially prevents the penetration of essential oils into the cell membrane (Chao *et al*., 2000; Burt, 2004; Mumivand *et al*., 2019). This confirms the antibacterial properties of *N. sativa* essential oil observed in the present and previous works.

**Table 2.** Minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) of *Nigella sativa* essential oil against food-borne bacteria.

| Pathogen | MIC (µg/mL) | MBC (µg/mL) |
|---|---|---|
| *Staphylococcus aureus* | 500 | 1,000 |
| *Bacillus cereus* | 500 | 1,000 |
| *Escherichia coli* | 500 | 2,000 |
| *Pseudomonas aeruginosa* | 500 | 2,000 |

*Physicochemical properties of cheese*

Based on Figure 1a, the effect of storage time on the amount of %dry matter was significant ($p < 0.05$), but the effect of concentration of *N. sativa* essential oil alone, and the interaction effect of essential oil concentration and storage time on the dry matter of cheese was not significant ($p > 0.05$). In a study conducted by Hamad *et al*. (2017), the results obtained was consistent with the present work, and it was found that the addition of essential oil had no significant effect on cheese dry matter. In a study by Hassanien *et al*. (2014), they achieved different results where the dry matter of cheese (control sample and samples with essential oil) gradually increased during storage. This could have been due to curd shrinking as a result of acidification.

Figure 1b shows that storage time had significant effect ($p < 0.05$) on the pH of cheese during the fermentation period. From this graph, it can be seen that as the fermentation time progressed, the pH in the samples containing the essential oil of *N. sativa* and the control sample decreased significantly. The pH in the control sample was 5.21 after one month, but this value reached 5.00 in the second month, and 4.81 in the third month. This was consistent with the results of the study by Hassanien *et al*. (2014). According to the study of El Soda *et al*. (1995), the main reason for the decrease in pH of cheese during ripening is due to the partial completion of lactose fermentation and the production of amino acids and lactic acid. The comparison between the control sample and the

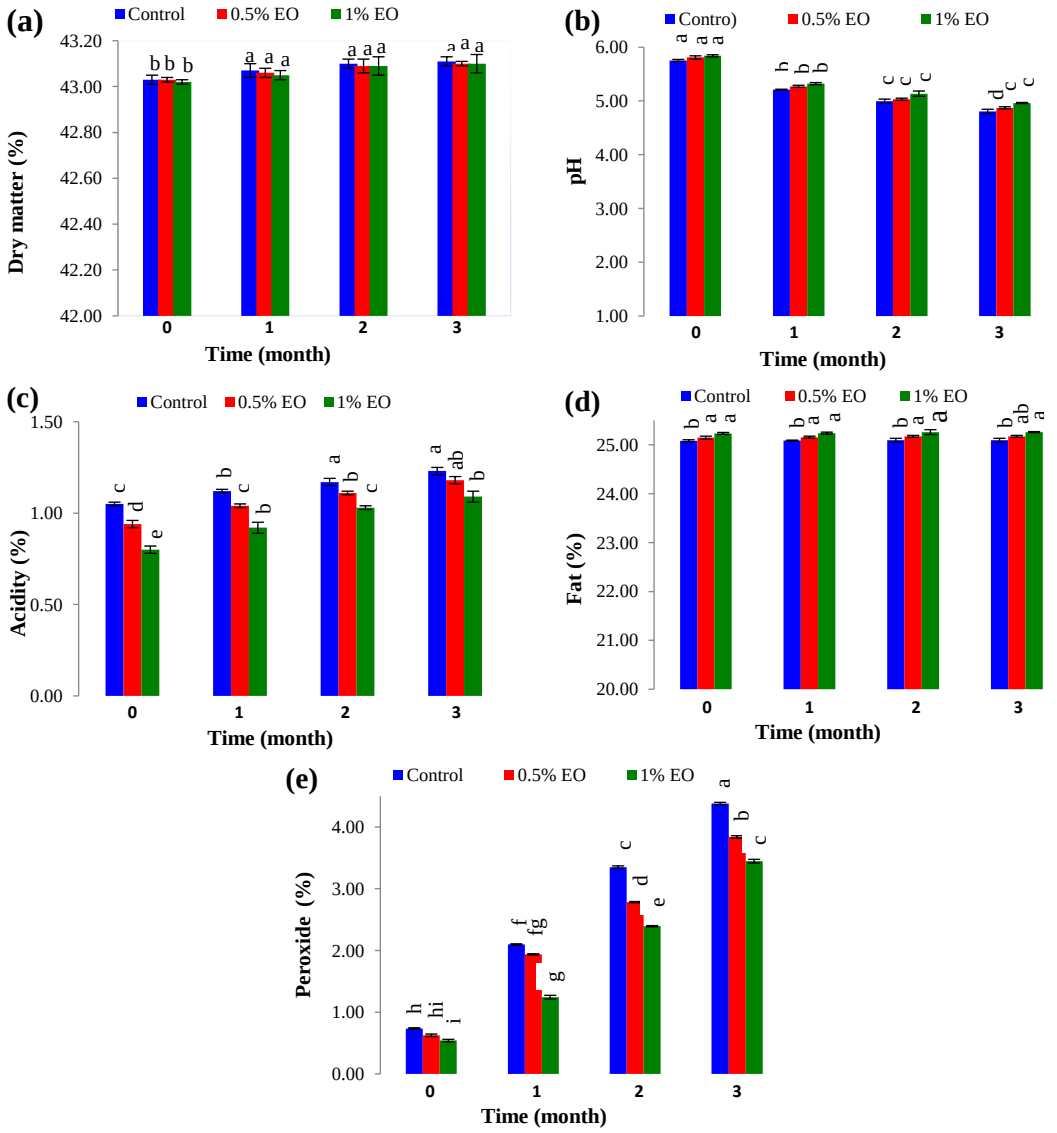


**Figure 1.** Effect of *Nigella sativa* essential oil concentrations and storage times on dry matter **(a)**, pH **(b)**, acidity **(c)**, fat **(d)**, and peroxide **(e)** of cheese. For each parameter, different lowercase letters in similar column indicate significant difference ($p < 0.05$). EO: essential oil.

samples containing essential oil of *N. sativa* in the present work showed that the essential oil had significant effect ($p < 0.05$) on the pH ($p < 0.05$) in the third month, and the pH of the samples containing essential oil was higher than that of the control sample. In the study of Arici *et al*. (2005), it was found that lactic acid bacteria (LAB) were more resistant to the essential oil of *N. sativa* than other Gram-positive bacteria. However, the essential oil had a small inhibitory effect on the growth of these bacteria, and therefore resulted in a small increase in pH compared to the control (Arici *et al*., 2005).

The effect of the addition of *N. sativa* essential oil on the average %acidity values during the storage period is shown in Figure 1c. It was apparent that the addition of *N. sativa* essential oil had significant effect ($p < 0.05$) on the acidity of cheese during the storage period. In months 0, 1, 2, and 3 of storage, a significant difference ($p < 0.05$) was found between the control sample and the other groups. In general, the acidity in the samples containing *N. sativa* essential oil and in the control sample increased significantly ($p < 0.05$) with increasing storage time. The lowest acidity in the sample containing 1% essential oil was observed on the first day, and the highest level in the control sample was observed in the third month of storage ($p < 0.05$). Based on these results, the acidity changes were in line with the pH changes during the ripening period in different cheese samples.

The results of the effect of the essential oil of *N. sativa* on the average fat content of cheese during the storage period are shown in Figure 1d which shows that changes in the percentage of essential oil had significant effect ($p < 0.05$) on the fat content of cheese, and the fat content in the samples with essential oil was higher than in the control sample. There was no significant difference between the fat contents of the groups in the different storage periods.

Figure 1e shows that changes in the percentage of *N. sativa* essential oil had significant effect ($p < 0.05$) on the amount of cheese peroxide. In general, with an increase in the percentage of essential oil, a decrease in the amount of cheese peroxide was observed. This agreed with Çakmakçi *et al*. (2014). The inhibition of peroxides and reduction of peroxide index is due to the antioxidant properties of compounds such as thymoquinone, carvacrol, and terpineol in *N. sativa* essential oil; the presence of these compounds is shown in Table 1. In a previous study, thymoquinone, carvacrol, and terpineol were shown to have remarkable radical scavenging properties (Burits and Bucar, 2000). The current findings were also in line with the results of El-Kholy and Aamer (2017) and Mahcene *et al*. (2021).

*Microbial characteristics of cheese*

The results showed that the changes in the total viable count of cheese were significant ($p < 0.05$) in different concentrations of *N. sativa* essential oil during storage (Figure 2a). As the concentration of *N. sativa* essential oil increased, the total bacterial population of the cheese decreased; the lowest total bacterial count was found in the samples with a concentration of 1% essential oil, and the highest content of the total bacterial population was found in the control sample. Considering the antibacterial properties of *N. sativa* essential oil mentioned earlier, these results were predictable. Abd Elmontaleb *et al*. (2020) added different concentrations of *N. sativa* essential oil to Edam cheese, and examined the total number of microorganisms. The total microbial count of the different groups decreased in all cheese samples containing essential oil; the highest total microbial count was observed in the control group in the last month. As a result, the concentration of essential oil in the samples increased, and the total microbial count decreased in the last month (Abd Elmontaleb *et al*., 2020). Badawi (2009) used the essential oil of *N. sativa* in the production of white cheese samples, and concluded that the use of this essential oil inhibited the growth of total viable count, coliform bacteria, and lipolytic and proteolytic bacteria. This agreed with the results observed in the present work. In the present work, the increase in the total number of microorganisms was lower in the sample with 1% essential oil concentration compared to the samples with 0.5% essential oil and the control, which was an indication of the greater microbial inhibitory effect of essential oil at higher concentrations. This increase in bacterial count during fermentation in all samples might have been due to the increase in the number of LAB. As mentioned earlier, these bacteria are more resistant to essential oils compared to other Gram-positive bacteria; they were able to continue to grow during fermentation (Arici *et al*., 2005; Gann, 2013), but the total bacterial count in samples containing essential oil was lower compared to the control sample. In general, essential oils can be used as natural

preservatives to extend the shelf life of food. For example, the essential oil of *Cuminum cyminum* L. decreased the total bacterial count in mayonnaise compared to the control sample (Moradi *et al*., 2023).

The changes in the fungal count of cheese in different concentrations of *N. sativa* essential oil during the storage period and their interaction were significant ($p < 0.05$) (Figure 2b). In general, with increasing storage time, the number of fungi significantly ($p < 0.05$) decreased in the samples containing *N. sativa* essential oil and in the control sample. The highest fungal count in the control sample was on the day of preparation, and the lowest fungal count in the sample containing 1% essential oil of *N. sativa* was in the third month. The pattern of reduction of fungal count during the storage period of foods containing essential oil was consistent with the results of Kouhi *et al*. (2020) and Moradi *et al*. (2023). Taherkhani *et al*. (2015), in a study investigating the effect of different concentrations of black cumin essential oil in the production of Gouda cheese, concluded that yeasts were very sensitive to essential plant oils, especially to the essential oil of *N. sativa*. Çakır and Çakmakçı (2018) investigated the effect of adding 1 and 2% essential oil of *N. sativa* on the number of microorganisms in Erzincan Tulum cheese. Their result was consistent with the present work. Similarly, Moradi *et al*. (2023) reported that the essential oil of *Cuminum cyminum* L. decreased the fungal count in mayonnaise compared to the control sample. The current findings also agreed with El-Kholy and Aamer (2017), Nunes Silva *et al*. (2020), Licon *et al*. (2020), and Mahcene *et al*. (2021).

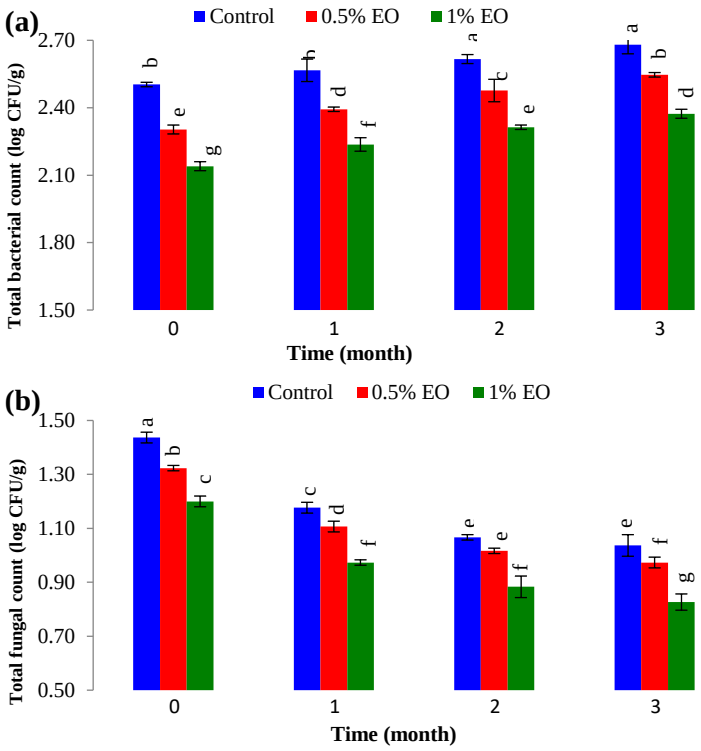


**Figure 2.** Effect of *Nigella sativa* essential oil concentrations and storage times on total bacterial count **(a)** and total fungal count **(b)**. For each parameter, different lowercase letters in similar column indicate significant difference ($p < 0.05$). EO: essential oil.

*Sensory characteristics of cheese samples*

In the present work, the effect of different concentrations of *N. sativa* essential oil on the characteristics of colour, taste, texture, and overall acceptability of cheese samples was investigated. The results of the sensory evaluation (Table 2) showed that the addition of essential oil had no significant effect on the colour parameter. The addition of the essential oil of *N. sativa* up to 0.5% increased the acceptance of taste and overall acceptability. A concentration of 1% of this essential oil decreased the acceptance of taste, colour, texture, and overall acceptability compared to the control sample ($p < 0.05$). Our results agreed with those of Moradi *et al*. (2023). They reported that increasing the concentration of *Cuminum cyminum* L. essential oil in mayonnaise decreased the sensory scores, resulting in unpleasant colour and odour. The addition of

essential oils to foods can have different effects on the sensory properties of foods. Cakir *et al*. (2016) showed that the addition of *N. sativa* to cheese improved the sensory properties of the cheese. Abd Elmontaleb *et al*. (2020) reported a concentration of 0.6% of *N. sativa* essential oil as the most suitable concentration for overall acceptability of Edam cheese. The current finding also agreed with El-Kholy and Aamer (2017) and Mahcene *et al*. (2021) (Table 3).

**Table 3.** Effect of *Nigella sativa* essential oil on sensory properties of cheese. Means with different lowercase superscripts in similar column are significantly different ($p < 0.05$).

| Formulation | Colour | Texture | Taste | Overall acceptability |
|---|---|---|---|---|
| Control group | $4.56 \pm 0.63^{a}$ | $4.25 \pm 0.32^{a}$ | $4.21 \pm 0.52^{b}$ | $4.12 \pm 0.47^{b}$ |
| Cheese + 0.5% *N. sativa* essential oil | $4.54 \pm 0.66^{a}$ | $4.12 \pm 0.38^{b}$ | $4.44 \pm 0.51^{a}$ | $4.34 \pm 0.52^{a}$ |
| Cheese + 1% *N. sativa* essential oil | $4.50 \pm 0.61^{a}$ | $4.08 \pm 0.43^{c}$ | $4.13 \pm 0.49^{c}$ | $4.00 \pm 0.49^{c}$ |

## Conclusion

The present work showed that the addition of *N. sativa* essential oil to cow's milk cheese not only controlled the growth of bacteria and fungi, but also reduced the oxidation of fats, thus delaying oxidative spoilage. Overall, 0.5% *N. sativa* essential oil would be the best concentration to extend the shelf life of cheese while improving taste acceptance and overall acceptability.

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