Effect of Nigella sativa (seed and oil) on the bacteriological quality of soft white cheese

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Abstract

The effect of Nigella sativa seed (1% and 3%) and oil (0.3% and 1%) on some food poisoning and pathogenic bacteria as well as on the total bacterial count TBC (cfu/g) in soft white cheese prepared from raw ewe's milk and laboratory pasteurized ewe's milk inoculated with Staphylococcus aureus, Brucella melitensis and Escherichia coli at a concentration of 1×10^5 cfu/ml were carried out. Cheese samples were examined for bacterial count at: zero, 2nd, 4th and 6th days of storage at refrigerator temp. Results showed that there was Significant decrease (P<0.05) in TBC, concentration dependent inhibition in contrast to control cheese samples which exerted significant increase in bacterial counts as it reached 2.8×10^6 cfu/g for TBC, Staph. aureus, Br. melitensis and E. coli respectively at the 6th day of storage at refrigerator temp. N. sativa oil (0.3% and 1%) was significantly more affective (P<0.05) as antibacterial agent than seed (1% and 3%) respectively. No significant differences (P>0.05) in the susceptibility of Staph. aureus, Br.melitensis and E. coli to the antibacterial effect of N. sativa seed (1% and 3%) and oil (0.3% and 1%) were observed in treated soft white cheese.

Keywords: Nigella sativa; Bacteriological quality; Soft white cheese.

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دراسة تأثير بذور وزيت الحبة السوداء Nigella sativa

على النوعية البكتيرولوجية للجبين الأبيض الطري

سناء داود الصواف ونورة صلاح الدين التعيمي

فرع الصحة العامة البيطرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

اختبرت الفعالية المضادة للجراثيم لبذور وزيت الحبة السوداء Nigella sativa على بعض أنواع الجراثيم المسببة للنسمة 알고راض Staph. aureus, Br. melitensis, E. coli في الجبن الأبيض الطري المصنوع من حليب التعاج الخام لفترات مختلفة من 1 إلى 6 أيام. أظهرت النتائج انخفاضاً معيناً في عدد الجراثيم الميكروباتية للبذور (TBC) عند دخولها بالكميات المعReserved. عند استخدام نسب مختلفة من بذور (1% و 3%) وزيت (0.3% و 1%) للجزء البشري لل식ية، مع خفيف. 3% و 1% من الخمير في درجة حرارة الثلاجة، أظهرت النتائج انخفاضاً معيناً في عدد الجراثيم الميكروباتية للبذور (TBC) عند دخولها بالكميات المعReserved. عند استخدام نسب مختلفة من بذور (1% و 3%) وزيت (0.3% و 1%) للجزء البشري لل식ية، مع خفيف. 3% و 1% من الخمير في درجة حرارة الثلاجة، أظهرت النتائج انخفاضاً معيناً في عدد الجراثيم الميكروباتية للبذور (TBC) عند دخولها بالكميات المعReserved. عند استخدام نسب مختلفة من بذور (1% و 3%) وزيت (0.3% و 1%) للجزء البشري لل식ية، مع خفيف. 3% و 1% من الخمير في درجة حرارة الثلاجة، أظهرت النتائج انخفاضاً معيناً في عدد الجراثيم الميكروباتية للبذور (TBC) عند دخولها بالكميات المعReserved. عند استخدام نسب مختلفة من بذور (1% و 3%) وزيت (0.3% و 1%) للجزء البشري لل식ية، مع خفيف. 3% و 1% من الخمير في درجة حرارة الثلاجة، أظهرت النتائج انخفاضاً معيناً في عدد الجراثيم الميكروباتية للبذور (TBC) عند دخولها بالكميات المعReserved. عند استخدام نسب مختلفة من بذور (1% و 3%) وزيت (0.3% و 1%) للجزء البشري لل식ية، مع خفيف. 3% و 1% من الخمير في درجة حرارة الثلاجة، أظهرت النتائج انخفاضاً معيناً في عدد الجراثيم الميكروباتية للبذور (TBC) عند دخولها بالكميات المعReserved. عند استخدام نسب مختلفة من بذور (1% و 3%) وزيت (0.3% و 1%) للجزء البشري لل식ية، مع خفيف. 3% و 1% من الخمير في درجة حرارة الثلاجة، أظهرت النتائج انخفاضاً معيناً في عدد الجراثيم الميكروباتية للبذور (TBC) عند دخولها بالكميات المعReserved. عند استخدام نسب مختلفة من بذور (1% و 3%) وزيت (0.3% و 1%) للجزء البشري لل식ية، مع خفيف. 3% و 1% من الخمير في درجة حرارة الثلاجة، أظهرت النتائج انخفاضاً معينةً في عدد الجراثيم الميكروباتية للبذور (TBC) عند دخولها بالكميات المعReserved. عند استخدام نسب مختلفة من بذور (1% و 3%) وزيت (0.3% و 1%) للجزء البشري لل식ية، مع خفيف. 3% و 1% من الخمير في درجة حرارة الثلاجة، أظهرت النتائج انخفاضاً معينةً في عدد الجراثيم الميكروباتية للبذور (TBC) عند دخولها بالكميات المعReserved. عند استخدام نسب مختلفة من بذور (1% و 3%) وزيت (0.3% و 1%) للجزء البشري لل식ية، مع خفيف. 3% و 1% من الخمير في درجة حرارة الثلاجة، أظهرت النتائج انخفاضاً معينةً في عدد الجراثيم الميكروباتية للبذور (TBC) عند دخولها بالكميات المعReserved. عند استخدام نسب مختلفة من بذور (1% و 3%) وزيت (0.3% و 1%) للجزء البشري لل식ية، مع خفيف. 3% و 1% من الخمير في درجة حرارة الثلاجة، أظهرت النتائج انخفاضاً معينةً في عدد الجراثيم الميكروباتية للبذور (TBC) عند دخولها بالكميات المعReserved. عند استخدام نسب مختلفة من بذور (1% و 3%) وزيت (0.3% و 1%) للجزء البشري لل식ية، مع خفيف. 3% و 1% من الخمير في درجة حرارة الثلاجة، أظهرت النتائج انخفاضاً معينةً في عدد الجراثيم الميكروباتية للبذور (TBC) عند دخولها بالكميات المعReserved. عند استخدام نسب مختلفة من بذور (1% و 3%) وزيت (0.3% و 1%) للجزء البشري لل식ية، مع خفيف. 3% و 1% من الخمير في درجة حرارة الثلاجة، أظهرت النتائج انخفاضاً معينةً في عدد الجراثيم الميكروباتية للبذور (TBC) عند دخولها بالكميات المعReserved. عند استخدام نسب مختلفة من بذور (1% و 3%) وزيت (0.3% و 1%) للجزء البشري لل식ية، مع خفيف. 3% و 1% من الخمير في درجة حرارة الثلاجة، أظهرت النتائج انخفاضاً معينةً في عدد الجراثيم الميكروباتية للبذور (TBC) عند دخولها بالكميات المعReserved. عند استخدام نسب مختلفة من بذور (1% و 3%) وزيت (0.3% و 1%) للجزء البشري لل식ية، مع خفيف. 3% و 1% من الخمير في درجة حرارة الثلاجة، A
Introduction

Soft white cheese locally made from sheep's and/or goat's milk are available in Mosul market during spring season. The cheese is made directly after milking, without any heat treatment. Thus, the traditional way of making this type of cheese lacks the simple hygienic measure even in the way for its display in local market. Frequently, some consumers suffer from diarrhea, gastrointestinal pain and brucellosis (1).

N. sativa seed (Black seed) is a plant which has been used for centuries for medicinal and culinary purposes and reported to possess a number of pharmacological properties, including antimicrobial activity (2).

Staph. aureus is a pathogenic bacteria which can affect any part of the body causing several diseases as septicemia, brain abscess, and enterocolitis (3). Because of the frequent contamination of milk from dairy personnel, and the high incidence of Staphylococcal mastitis in dairy herds, the most commonly occurring type of food poisoning is due to enterotoxin-producing strains of Staph. aureus (4).

Br. melitensis is the most common species in the genus Brucella in several parts of the world (5). Br. melitensis can infect human and animal causing brucellosis which is a systemic disease which can cause different clinical manifestations (6). The danger of contracting brucellosis through the consumption of unpasteurized raw milk and dairy products drew the attention of public health authorities in many countries to impose quarantine regulations governing the consumption of such products (7).

E. coli also is a food pathogen, strains of this species express potent toxins and cause serious gastrointestinal infections. Additionally it can result in life-threatening systemic disease (8).

Although optimum growth temp. is 37°C for Staph. aureus, Br. melitensis and E. coli, Staph. aureus can grow at temperatures slightly above 5°C, Br. melitensis can survive at 5°C for long periods of time and are part of a so-called "new problem" with dairy products, while E. coli exhibit competitive growth in milk at 5°C (9).

Therefore, this work was planned to build up information on the effect of N. sativa seed and oil on growth and survival of total bacteria, Staph. aureus, Br. melitensis and E. coli in soft white cheese during manufacturing and storage at refrigerator temp.

Materials and methods

The bacteria used in this study are known to be the cause of disease in human and some are known to be involved in food poisoning.

Staph. aureus was obtained from Mr. Omar Hashem Sheet, Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul.

Br. melitensis was obtained from Miss Amera Ali Ahmed, Department of Basic Science, Nursing College, University of Mosul.

E. coli was obtained from Mrs. Noor Abd Alhafez Jerjees, Department of Biology, Education College, University of Mosul.

Each isolate was confirmed by reculturing on the selective media and by performing the biochemical tests (10).

Staph. aureus and E. coli were propagated in Nutrient broth at 37°C for 24h. Two transfers were made prior to inoculation, the cfu/ml was determined using Mannitol Salt Agar and MacConkey Agar as a selective media for Staph. aureus and E. coli, respectively.

Br. melitensis was propagated in Brucella broth at 37°C for 48h. Also two transfers were made prior to inoculation, the cfu/ml was determined using Brucella Agar as a selective medium (11).

The procedure described by (12) was used for preparation of soft white cheese.

For total bacterial count (TBC), raw ewe's milk was used for preparation of soft white cheese. For other treatments raw ewe's milk was laboratory pasteurized at 63°C for 30 min. Pasteurized milk was divided into three categories, each category was inoculated with one type of bacteria under study (Staph. aureus, Br. melitensis and E. coli) to yield a concentration of about 1x10⁶ cfu/ml. Samples from raw and inoculated milk were taken to determine the initial count of bacteria (11).

Raw and each category of inoculated milk were divided into five equal parts. The first one was considered as a control, while the 2nd and 3rd parts were mixed with 1% and 3% of N. sativa seed and the 4th and 5th parts were mixed with 0.3% and 1% of N. sativa oil (cold pressed). These concentrations of N. sativa seed and oil were used as 1% and 3% of N. sativa seed approximately contain 0.3% and 1% of its oil respectively.

Samples from finished cheese were examined for TBC, Staph. aureus, Br. melitensis and E. coli count. Finished
cheese were stored at refrigerator temp. (5±2°C). Sampels from the storage cheese were examined in the 2nd, 4th and 6th days of storage for count of mentioned bacteria according to (11).

The data were Statistically analyzed using Sigma Stat software Version 3.10 (2004). Analysis of variance procedures appropriate for either a two-way completely randomized design for data involved in the effect of different concentrations of bacterial inhibition materials (N. sativa seed and oil) and storage durations and their interaction and a one-way completely randomized design for data concerned with degree of bacterial sensitivity to each concentration of the inhibitor, according to (13). Significant differences (P<0.05) among treatment means were detected based on Duncan Multiple Range Test (14).

Results

The results illustrated in Table (1) verify that there was significant increase (P<0.05) in TBC in finished (zero day) and stored (2nd, 4th and 6th days of storage at refrigerator temp.) cheese (control) as it reached 2.8×10^6 cfu/g at the 6th day of storage. Also significant differences (P<0.05) in TBC between control (continue increasing) and treated cheese samples were observed. Cheese samples made from milk treated with 1% (1st treatment) and 3% (2nd treatment) of N. sativa seed and 0.3% (3rd treatment) and 1% (4th treatment) of N. sativa oil also showed an increase in TBC in the finished cheese with a minimum increase (6.2×10^6 cfu/g) was reported in the 4th treatment. This increment in counts was significantly different (P<0.05) between treatments, except 2nd and 3rd treatments were not significantly different (P<0.05). Results referred that there was a significant decrease (P<0.05) in TBC in treated cheese with N. sativa seed and oil during storage at refrigerator temperature, until it reached 1.95×10^6, 1.2×10^6, 1.34×10^6 and 9.4×10^5 cfu/g in the 1st, 2nd, 3rd and 4th treatments, respectively at the 6th day of storage with the exception of 4th treatment during the 4th and 6th days of storage which the decrease in TBC was not significantly different (P<0.05). Significant differences (P<0.05) in TBC between treatments at the 2nd day of storage were observed. But no significant differences (P<0.05) between the 1st and 2nd treatments and 2nd and 3rd treatments were recorded. During the 4th and 6th days of storage also there was significant differences (P<0.05) in TBC between treatments except between 1st and 3rd treatments and 2nd and 3rd treatments at the 4th day of storage and between 1st and 3rd treatments and 2nd, 3rd and 4th treatments at the 6th day of storage where TBC were not significantly different (P<0.05). Regardless of storage period results showed significant differences (P<0.05) in TBC between control and between treatments with the exception of 2nd and 3rd treatments which was not significantly different (P<0.05).

The corresponding results obtained from cheese prepared from milk inoculated with Staph. aureus at a level of 1×10^9 cfu/ml (control) and stored at refrigerator revealed that Staph. aureus count significantly increased (P<0.05) from 2.7×10^6 cfu/g in the finished cheese (zero day) to 2.95×10^6 cfu/g at the 6th day of storage. It was obvious that there was significant differences (P<0.05) in Staph. aureus counts between control and N. sativa seed and oil treated cheese samples in the finished cheese and during its storage at refrigerator temp. Firstly Staph. aureus counts increased in finished cheese prepared from milk inoculated with the bacteria and treated with N. sativa seed and oil with a minimum increase (1.93×10^6 cfu/g) observed in the 4th treatment. Significant differences (P<0.05) in Staph. aureus counts between treatments were recorded at zero time except between 1st and 2nd treatments and between 2nd and 3rd treatments which increment in counts was not significantly different (P<0.05). After that, Staph. aureus counts significantly decreased (P<0.05) in treated cheese with N. sativa seed and oil during storage at refrigerator temp. - in contrast to the control cheese samples- as it reached 2.57×10^6, 1.5×10^6, 1.74×10^6 and 8.5×10^5 cfu/g in the 1st, 2nd, 3rd and 4th treatments, respectively at the 6th day of storage except 4th treatment at the 4th and 6th days of storage where decrease in counts was not significantly different (P<0.05). At the 2nd and 4th day of storage results showed significant differences (P<0.05) in Staph. aureus counts between treatments with the exception of 1st and 3rd treatments and 2nd and 3rd treatments which differences were not significant (P<0.05). No significant differences (P<0.05) in Staph. aureus counts were observed between treatments at the 6th day of storage of treated cheese except 1st and 4th treatments which Staph. aureus count was significantly different (P<0.05). As mentioned in table (1), results showed that there was significant differences (P<0.05) in counts of Staph. aureus between control and between treatments regardless of storage period. But no significant differences (P>0.05) in counts between 2nd and 3rd treatments was noticed (Table 2).

From data in Table (3) it was evident that Br. melitensis counts increased as it reached 2.23×10^6 cfu/g at the 2nd and 4th days of storage, then slightly declined to reach 2.22×10^6 cfu/g at the 6th day of storage of control cheese samples prepared from milk inoculated with Br. melitensis at a conc. of 1×10^6 cfu/ml. This increment and decline in counts of Br. melitensis was not significant (P>0.05), where as there was significant differences (P<0.05) in Br. melitensis counts between control and treated cheese samples in the finished cheese and during storage at refrigerator temp. Br. melitensis counts also increased in the finished cheese prepared from milk inoculated with the bacteria and treated with N. sativa seed and oil to record a minimum increase in counts (1.29×10^6 cfu/g) in the 4th treatment at zero time. Br. melitensis counts were significantly different (P<0.05)
between treatments at zero time. During storage at refrigerator temp., results showed significant decrease (P<0.05) in counts of *Br.melitensis* in treated cheese sampels until it reached $4.5 \times 10^5$, $2.9 \times 10^5$, $3.2 \times 10^5$ and $2.2 \times 10^5$ cfu/g in the 1st, 2nd, 3rd and 4th treatments, respectively at the 6th day of storage. Significant differences (P<0.05) in *Br.melitensis* counts were observed between treatments of cheese at each storage period in refrigerator, except between 1st and 3rd treatments, 2nd and 3rd treatments and 2nd, 3rd and 4th treatments at the 2nd, 4th and 6th day of storage, respectively where differences in counts were not significant (P<0.05). Control and treated cheese sampels showed significant differences (P<0.05) in *Br.melitensis* counts between them, regardless of storage period.

Table 1: Effect of *Nigella sativa* (seed and oil) on the means of TBC (cfu/g) during manufacturing and storage of soft white cheese at refrigerator temp.

<table>
<thead>
<tr>
<th>Storage Period (days)</th>
<th>Control</th>
<th>1% Seed</th>
<th>3% Seed</th>
<th>0.3% Oil</th>
<th>1% Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$1.8 \times 10^7$</td>
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<td>$7.4 \times 10^6$</td>
<td>$7.6 \times 10^6$</td>
<td>$6.2 \times 10^6$</td>
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<td>$2.2 \times 10^7$</td>
<td>$4.7 \times 10^6$</td>
<td>$4.1 \times 10^6$</td>
<td>$3.8 \times 10^6$</td>
<td>$2.8 \times 10^6$</td>
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<tr>
<td>4</td>
<td>$2.4 \times 10^7$</td>
<td>$3 \times 10^6$</td>
<td>$2.2 \times 10^6$</td>
<td>$2.5 \times 10^6$</td>
<td>$1.5 \times 10^6$</td>
</tr>
<tr>
<td>6</td>
<td>$2.8 \times 10^7$</td>
<td>$1.95 \times 10^6$</td>
<td>$1.2 \times 10^6$</td>
<td>$1.34 \times 10^6$</td>
<td>$9.4 \times 10^5$</td>
</tr>
<tr>
<td>Treatments effect</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>C</td>
<td>D</td>
</tr>
</tbody>
</table>

- Horizontally different small letters within each storage period are significantly different (P<0.05).
- Vertically different small letters within each treatment are significantly different (P<0.05).
- Different capital letters within last raw (treatments regardless of storage period) are significantly different (P<0.05).

Table 2: Effect of *N. sativa* (seed and oil) on the means of *Staphylococcus aureus* (cfu/g) during manufacturing and storage of soft white cheese at refrigerator temp*.

<table>
<thead>
<tr>
<th>Storage Period (days)</th>
<th>Control</th>
<th>1% Seed</th>
<th>3% Seed</th>
<th>0.3% Oil</th>
<th>1% Oil</th>
</tr>
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<tbody>
<tr>
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<td>$8.2 \times 10^5$</td>
<td>$6 \times 10^5$</td>
<td>$6.9 \times 10^5$</td>
<td>$3.2 \times 10^5$</td>
</tr>
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<td>$1.7 \times 10^5$</td>
</tr>
<tr>
<td>6</td>
<td>$2.95 \times 10^6$</td>
<td>$2.57 \times 10^5$</td>
<td>$1.5 \times 10^5$</td>
<td>$1.74 \times 10^5$</td>
<td>$8.5 \times 10^4$</td>
</tr>
<tr>
<td>Treatments effect</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>C</td>
<td>D</td>
</tr>
</tbody>
</table>

- Horizontally different small letters within each storage period are significantly different (P<0.05).
- Vertically different small letters within each treatment are significantly different (P<0.05).
- Different capital letters within last raw (treatments regardless of storage period) are significantly different (P<0.05).

* Bacteria was added to the milk used for preparation of soft white cheese at a conc. of $1 \times 10^6$ cfu/ml.

Results recorded in Table (4) revealed that *E. coli* counts increased significantly (P<0.05) from $2.585 \times 10^6$ cfu/g in the control cheese samples at zero time to $2.885 \times 10^6$ cfu/g at the 6th day of storage of cheese prepared from milk inoculated with *E. coli* at a level of $1 \times 10^6$ cfu/ml. *E. coli* counts were significantly different (P<0.05) between control and treated cheese samples in the finished cheese at zero time and during storage at refrigerator temp. As in control cheese sampels, at beginning *E. coli* counts increased in the finished cheese prepared from milk inoculated with the bacteria and treated with *N. sativa* seed (1% and 3%) and oil (0.3% and 1%) to record a minimum
increase (1.45×10^6 cfu/g) in the 3rd treatment at zero time. E. coli count was only significantly different (P<0.05) between 1st and 3rd treatments and 1st and 4th treatments at zero time. Later, E. coli counts significantly decreased (P<0.05) during storage of N. sativa seed and oil treated cheese at refrigerator temp, as it reached 7×10^5, 4.5×10^5 and 2.5×10^5 cfu/g in the 1st, 2nd, 3rd and 4th treatments, respectively at the 6th day of storage. But no significant decrease (P<0.05) was observed in the 1st, 2nd, 3rd and 4th treatments at the 6th day of storage. Every storage period showed no significant differences (P<0.05) in E. coli counts between treatments with the exception of 1st and 4th treatments at the 2nd day of storage, 1st and 3rd treatments and 1st and 4th treatments at the 4th day of storage, and 1st and 4th treatments at the 6th day of storage where differences in counts were significant (P<0.05). As mentioned in Table 1 and 2 significant differences (P<0.05) in E. coli counts between control and between treated cheese samples were observed, regardless of storage period, except 2nd and 3rd treatments which difference was not significant (P<0.05).

Table 3: Effect of N. sativa (seed and oil) on the means of Brucella melitensis (cfu/g) during manufacturing and storage of soft white cheese at refrigerator temp*.

<table>
<thead>
<tr>
<th>Storage Period (days)</th>
<th>Control</th>
<th>1% Seed</th>
<th>3% Seed</th>
<th>0.3% Oil</th>
<th>1% Oil</th>
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<td>1.41×10^6</td>
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<td>b</td>
<td>c</td>
<td>d</td>
<td>e</td>
<td></td>
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<td>2.23×10^6</td>
<td>1.12×10^6</td>
<td>8.9×10^5</td>
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</tr>
<tr>
<td>a</td>
<td>g</td>
<td>h</td>
<td>g</td>
<td>i</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.23×10^6</td>
<td>7.5×10^6</td>
<td>5.1×10^5</td>
<td>5.9×10^5</td>
<td>3.6×10^5</td>
</tr>
<tr>
<td>a</td>
<td>k</td>
<td>m</td>
<td>m</td>
<td>n</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.22×10^6</td>
<td>4.5×10^6</td>
<td>2.9×10^5</td>
<td>3.2×10^5</td>
<td>2.2×10^5</td>
</tr>
<tr>
<td>a</td>
<td>p</td>
<td>q</td>
<td>q</td>
<td>q</td>
<td></td>
</tr>
<tr>
<td>Treatments effect</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
</tr>
</tbody>
</table>

- Horizontally different small letters within each storage period are significantly different (P<0.05).
- Vertically different small letters within each treatment are significantly different (P<0.05).
- Different capital letters within last raw (treatments regardless of storage period) are significantly different (P<0.05).
* Bacteria was added to the milk used for preparation of soft white cheese at a conc. of 1×10^6 cfu/ml.

Table 4: Effect of N. sativa (seed and oil) on the means of E. coli (cfu/g) during manufacturing and storage of soft white cheese at refrigerator temp*.

<table>
<thead>
<tr>
<th>Storage Period (days)</th>
<th>Control</th>
<th>1% Seed</th>
<th>3% Seed</th>
<th>0.3% Oil</th>
<th>1% Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.585×10^6</td>
<td>1.85×10^6</td>
<td>1.65×10^6</td>
<td>1.45×10^6</td>
<td>1.55×10^6</td>
</tr>
<tr>
<td>a</td>
<td>b</td>
<td>bc</td>
<td>c</td>
<td>c</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.735×10^6</td>
<td>1.1×10^6</td>
<td>8.5×10^5</td>
<td>8.5×10^5</td>
<td>6×10^5</td>
</tr>
<tr>
<td>a</td>
<td>e</td>
<td>ef</td>
<td>ef</td>
<td>fi</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.83×10^6</td>
<td>8.5×10^6</td>
<td>6.5×10^5</td>
<td>5.5×10^5</td>
<td>4×10^5</td>
</tr>
<tr>
<td>aj</td>
<td>h</td>
<td>hif</td>
<td>ik</td>
<td>im</td>
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</tr>
<tr>
<td>6</td>
<td>2.885×10^6</td>
<td>7×10^5</td>
<td>4.5×10^5</td>
<td>4.5×10^5</td>
<td>2.5×10^5</td>
</tr>
<tr>
<td>j</td>
<td>kh</td>
<td>kmi</td>
<td>km</td>
<td>m</td>
<td></td>
</tr>
<tr>
<td>Treatments effect</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
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</tr>
</tbody>
</table>

- Horizontally different small letters within each storage period are significantly different (P<0.05).
- Vertically different small letters within each treatment are significantly different (P<0.05).
- Different capital letters within last raw (treatments regardless of storage period) are significantly different (P<0.05).
* Bacteria was added to the milk used for preparation of soft white cheese at a conc. of 1×10^6 cfu/ml.
The information obtained by the achieved results in Table (5) proved that there was no significant differences (P<0.05) in the mean values of examined bacterial counts (Staph. aureus, Br. melitensis, and E. coli) within the same treatment in cheese samples prepared from milk inoculated with these bacteria and treated with N. sativa seed (1% and 3%) and oil (0.3% and 1%) after 6th days of storage at refrigerator temp.

Table 5: Comparison of the antibacterial activity of N. sativa seed and oil towards bacterial types under study in soft white cheese.

<table>
<thead>
<tr>
<th>Bacterial type</th>
<th>Mean ± Se</th>
<th>1% Seed</th>
<th>3% Seed</th>
<th>0.3% Oil</th>
<th>1% Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph. aureus</td>
<td></td>
<td>7.8675×10^3 ±</td>
<td>6.4×10^3 ±</td>
<td>6.76×10^3 ±</td>
<td>4.125×10^3 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.8658×10^3 ±</td>
<td>1.96086×10^3 ±</td>
<td>1.72758×10^3 ±</td>
<td>1.66539×10^3 ±</td>
</tr>
<tr>
<td>Br. melitensis</td>
<td></td>
<td>1.0425×10^6 ±</td>
<td>7.75×10^3 ±</td>
<td>8.9×10^3 ±</td>
<td>6.25×10^3 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.98104×10^5 ±</td>
<td>1.60732×10^5 ±</td>
<td>1.82795×10^5 ±</td>
<td>1.55878×10^5 ±</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
<td>1.125×10^6 ±</td>
<td>9×10^3 ±</td>
<td>8.25×10^3 ±</td>
<td>7×10^3 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.70115×10^5 ±</td>
<td>1.75895×10^5 ±</td>
<td>1.50641×10^5 ±</td>
<td>1.92251×10^5 ±</td>
</tr>
</tbody>
</table>

- Vertically similar letters are not significant (P<0.05).

Discussion

It was clear from the forementioned results there was an obvious increase in bacterial counts in finished cheese at zero time (Tables 1-4), this increment primarily belong to that all the organisms present in the milk become part of the fresh curd flora, being concentrated in the curd (4).

It could be noticed the high counts of the bacterial types (TBC, Staph. aureus, Br.melitensis and E. coli) in control soft white cheese in contrast to the rapid reduction of bacterial counts in cheese containing N. sativa seed and oil added during preparation. Results are in agreement with those observed by (15) who showed similar antibacterial activity of N. sativa seed on Staph. aureus in Domiati cheese and with the results obtained by (16) who mentioned that E. coli was not detectable after three and two weeks of storage at room and refrigerator temperatures respectively for cheeses prepared from milk inoculated with the bacteria (2×10^6 cfu/ml) and treated with 5% and 10% of sod. chloride and N. sativa seeds at a concentration of 1% and 3%. Also the results agree to a certain extent with those reported by (17) who indicated that adding of N. sativa seed at a conc. of 1% to the processed cheese inhibited microbial growth and with the results recorded by (18) who noticed an inhibitory effect of essential oil of N. sativa on microorganisms especially on the total number of bacteria, coliform bacteria, lipolytic and proteolytic bacteria during storage of soft white cheese treated with the oil at a ratio of 0.5% of curd weight at 5˚C for 28 days. Similar inhibitory influence induced by N. sativa on microorganisms in yoghurt was reported by (19) who showed that addition of alcoholic extract of black seeds in a conc. of 3% was caused prolongation of its keeping quality from 7 to 14 days. Another study (20) indicated that milk treatment with oil of black seed (0.5%) gave good results for holding the raw milk quality in acceptable level for 19 hours at temp. 25˚C and for 5 days at 5˚C. The results of water extract of N. sativa seed treatment (0.5%) and the oil treatment (0.2%) showed less shelf time 17 hours at 25˚C and 4 days at 5˚C while water extract treatment at a conc. of 0.2% gave an acceptable result in the milk quality preservation 11 hours at 25˚C and 3 days at 5˚C comparable with control which gave shelf time 13 hours at room temp. and 1 day at 5˚C. Finally the investigator recomended the use of water extract and oil of N. sativa as natural preservative materials for raw milk.

Several studies in vitro referred to the antibacterial effect of extracts and oil of black seed against Staph. aureus and E. coli (2,21-24). A great inhibitory effect of concentrated crude aqueous extract of N. sativa seed on Brucella bacteria was reported by (25). Whereas (26) recorded a slight antibacterial effect of N. sativa oil on E. coli while aqueous and alcoholic extracts showed no effect on this bacteria.

Our results are in agreement with those obtained by (2,27) who showed pronounced concentration dependent inhibition of all the bacteria tested (Table 1-4).

The present findings in Table 1-4 revealed that N. sativa oil (0.3% and 1%) was significantly more effective (P<0.05) as antibacterial agent than seed (1% and 3%) respectively. This is primarily belongs to the presence of Thymoquinone TQ (2-isopropyl-5-methyl-benzoquinone) which considered as one of the major components of N. sativa volatile oil, but which is also present in the fixed oil (28,29). Antibacterial effect of TQ was due to the inhibition of RNA and protein synthesis (22), as well as α-Finene (The unsaturated bicyclic monoterpen hydrocarbon) which
also present in *N. sativa* volatile oil and exerts antibacterial action (30). So bacterial types present in cheeses treated with *N. sativa* oil will be more exposed to the antibacterial action than those present in *N. sativa* seed treated cheeses.

Results showed no significant differences (P<0.05) between test bacteria in their susceptibility to the antibacterial effect of *N. sativa* seed and oil (Table 5). These results were in contrast to those indicated that the antibacterial activity of *N. sativa* seed and oil was more against gram +ve bacteria than gram –ve bacteria (2,24). This perhaps because those studies were performed in plates (in vitro), while our research included study the antibacterial effect of *N. sativa* seed and oil in cheese prepared from milk inoculated with the test bacteria and treated with it. Cheese constituents may affect on the susceptibility of bacteria to seed and oil of *N. sativa* as sod. chloride at a conc. of 5% was added during manufacturing of soft white cheese, this may adversely affect on growth of *Br. melitensis* and *E. coli* and then increase susceptibility of these bacteria to the *N. sativa* seed and oil (16,31) comparable to *Staph. aureus* which can grow at high concentrations of NaCl may reach 10% (32).

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