Effect of thyme oil and nisin on *Bacillus cereus* in chicken fillet

Sara M. Haraz1, AboBaker M. Edris2, Radwa A. Lela3, Walid S. Arab2

1Director of Veterinary Medicine, El-Ghurbia, Egypt
2Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University.
3Department of Food Hygiene and Control, Animal Health Research Institute, Tanta branch

**ABSTRACT**

Extension of the shelf-life stability and food safety is the major goal recommended for chicken fillet processing. A total of 2100 g fresh chicken fillet were divided into 7 equal groups (3×100 g per each). The 1st group was negative control and other six group were inoculated by *Bacillus cereus* with an infective dose 5.2×10⁴ cfu/g and treated with thyme oil (1.0 % and 1.5%), nisin (50 ppm and 100 ppm) and mixture of them at (1.5% and 100 ppm). All chicken fillet samples were stored at 4℃ in refrigerator. The groups were examined every 48 hours for sensory examination (overall acceptability) and *Bacillus cereus* count. The experiment was performed in triplicate. Thyme oil (1% and 1.5%) decreased the count of *Bacillus cereus* with reduction percentage 21.2% and 53.8 % on the 4th day and 6th day of storage, respectively. Nisin (50 and 100 ppm) decreased the count of *Bacillus cereus* with reduction percentages 86.7% and 93.1% on the 8th day and 10th day of storage, respectively. Mixture of (thyme and nisin) decreased the count of *Bacillus cereus* with reduction percentage 98.5% and 100% on the 10th and 12th day of storage, respectively. Generally, mixture of thyme oil and nisin (1.5% and 100 ppm) proved to be more efficient than other concentrations in suppression of *Bacillus cereus* growth in chicken fillet and also showed overall acceptability until the 12th day of storage. Therefore, it is recommended to improve shelf-life and safety of the fresh chicken fillet.

1. **INTRODUCTION**

Chicken and chicken products provides protein of high biological value for consumers, where they contain all the essential amino acids with high proportion of unsaturated fatty acids and low cholesterol value. Chicken meat is a good source of different types of vitamins and minerals like vit A, thiamine, iron, and phosphorus. This places chicken meat as healthy food (Zaki and Shehata, 2008; Koblitz, 2011).

*Bacillus cereus* food poisoning is a major concern worldwide. This bacterium is an aerobic, spore forming bacterium and commonly found in soil. It can also be isolated from raw meat, processed foods and vegetables, and enters into the food chain either through contaminated food or water. Food poisoning from the past outbreaks include boiled and fried rice, vegetables, cooked meats, soups, and raw vegetable sprouts (FDA, 2012).

Spoilage of fresh chicken cuts is an economic burden to producers and requires investigating new methods to prolong their shelf life and the overall quality of the chicken, which is the chief problem faced in the poultry processing industry as well as consumers prefer (Burt, 2004). So, attention has been focused on herbs and spices extracts which have been used to improve the sensory characteristics and shelf-life of foods for centuries (Fernandez-Gines et al., 2005).

The antimicrobial activities of essential oils (thyme oil) are correlated to the presence of their bioactive volatile components (Mahmoud and Croteau, 2002). Essential oil from herbs and spices have been used to improve the sensory, characteristics and extend the shelf-life of foods (Gibbons, 2008). Compounds with phenolic groups are most effective against microbial population (Dorman and Deans, 2000).

Thyme is the major components of Thymus vulgaris oil and extract are thymol, π-cymene, carvacrol and γ-terpinene. They show very strong antibacterial, antifungal and antioxidant activities. Thyme essential oils retard food spoilage and increase the shelf life of foods (Mandal and Debmandal, 2016).

Nisin is the one of internationally accepted as a safe and preservative in certain foods for several decades, decreasing the risk for their transmission through the food chain. It is extremely resistant to heat, soluble in dilute acids and stable to boiling in such solutions. It is not toxic, even at levels much higher than those used in foods (Papagianni and Anastasiadou, 2009).

Nisin also inhibits the outgrowth of bacterial spores, including spores of *Bacillus cereus* involving either covalent binding to a spore target or loss of membrane integrity (Ian et al., 2011).

The goal of this research is to study the acceptability and the antimicrobial effect of thyme oil, nisin and mixture of them on *Bacillus cereus* inoculated in chicken fillet during refrigerated storage (4℃).

---

1 Correspondence to: saraharaz512@gmail.com
2. MATERIAL AND METHODS

2.1. Preparation of fresh chicken fillet samples:
A total amount of 2100 g of raw chicken fillet samples were purchased from a butcher’s shop, were placed in sterile polyethylene bags.
The examined chicken fillet samples were divided to 7 equal groups (3 x100 gm for each), first groups were control negative (no B. cereus was isolated), second group used as control positive, third and fourth groups were treated with Thyme oil (1% -1.5%), respectively. Fifth and six groups were treated with Nisin (50 ppm- 100 ppm), respectively, while the seven groups treated with mixture of both (thyme 1.5% and Nisin 100 ppm) as a mixture group.

2.2. Preparations of inoculum:
Bacillus cereus strain was obtained from Animal Health Research Institute (AHRI), Dokki, with recommended dose (5.2 x10³ CFU/ml) as recorded by McFarland’s nephelometer standards according to Slabyj et al. (2003).

2.2.1. Enrichment of B. cereus (Rahimi et al., 2013)
The collected samples were transferred instantly under complete aseptic conditions for bacteriological isolation and identification of B. cereus. Briefly, 25 grams of fresh chicken fillet were transferred to 225 ml of 0.1% sterile buffered peptone water then stomached for 2 minutes to provide a homogenate, then decimal serial dilution were prepared.

2.2.2. Selective plating of B. cereus (ISO, 2006)
A Loopful of serial dilution was seeded into the surface of Mannitol- Egg Yolk-Polymyxin Agar (MYP). The inoculum was spread over the entire surface of the agar with a sterile bent glass rod and the plates were inverted and incubated at 37°C for 48 hours, then examined for typical colonies of Bacillus cereus (pink colony with halo surrounded by a zone of egg yolk precipitation). The plates were re-incubated for further 48 hours in order to detect all the B. cereus colonies. Suspected colonies were picked up and subculture on nutrient broth and incubated at 37°C for 48 hours, then refrigerated at 4°C for further biochemical identification.

2.3. Thyme preparation
Thyme oil was prepared by hydro-distillation method and provided by Agriculture Research Center, Egypt. Tween 80 was added, as diluent for even distribution and dissolving, to essential oils before applying on examined samples According to Wilkinson et al. (2003).

2.4. Nisin preparation
Nisin was prepared at concentrations 50 and 100 ppm according to Hassan (1999)

2.5. Experimental application
- Seven groups of fresh chicken fillet groups were inoculated with B. cereus (5.2x10³ cfu/ml) (Slabyj et al., 2003) were arranged as follows
  - Control –ve as first group (not inoculated with B. cereus and not treated with natural or chemical additives).
  - Control +ve as 2nd group (inoculated with B. cereus only and not treated with natural and chemical additives).
  - 3rd group inoculated with B. cereus treated with thyme 1%.
  - 4th group inoculated with B. cereus and treated with thyme 1.5%.
  - 5th group inoculated with B. cereus and treated with nisin 50 ppm.
  - 6th group inoculated with B. cereus and treated with nisin 100 ppm.
  - 7th group inoculated with B. cereus and treated with (thyme 1.5% + nisin 100 ppm).

All the examined sample groups were packed in separate sterile polyethylene bags and stored in domestic refrigerator at nearly ±4 °C. Each sample was analyzed at Day zero, 2nd, 4th, 6th, 8th, 10th and 12th during storage. This experiment was conducted in triplicate.

2.5. Sensory examination
Color, odor, and overall acceptability were determined for each sample of fresh chicken fillet according to Pearson and Tauber (1984).

Score System for Sensory Evaluation was Excellent at point (9) means bright red color, fleshy odor and firm, tender in consistency, Very very good at point (8), very good at point (7), Good at point (6), Medium at point (5), Fair at point (4), Poor at point (3), Very poor at point (2) and very very poor at point (1) means grey to greenish color, rancid and putrid odor and softness and slimminess in consistency.

3. RESULTS

Regarding the results recorded in table 1 all samples (treated and control) showed excellent score at zero day of treatment. On the 2nd day the control negative was medium and control positive was very very poor, but the other treated groups were very good, then decrease over all acceptability. On the 4th day, the control positive group decomposed, the control negative group score started to decrease with very very poor score while the other treated groups showed very good to good overall acceptability though samples treated with thyme 1.5% and nisin 100 ppm showed very very good acceptability. On the 6th day the control negative group decomposed, while thyme 1.5%, nisin 50 ppm, 100 ppm had good over all acceptability while mixture (1.5% and 100 ppm) had very good score of over-all Acceptability. On the 8th day samples treated with thyme oil (1%) was decomposed, while treated with 1.5 % was medium, nisin 50 ppm was fair, but mixture was good over-all acceptability. On the 10th day of treatment thyme 1.5%, nisin 50 ppm were decomposed, while nisin 100 ppm was medium and the mixture was good overall acceptability. At the last day (12th day) the 100 ppm group was decomposed while mixture treated samples showed fair over-all acceptability.
The results recorded in table 2 and 3 indicated that thyme oil (1%) reduced B. cereus count (cfu/g) artificially inoculated into fresh chicken fillet samples from 5.2±1.3 x10⁷ to 4.60±0.67x10⁷ and 4.10±0.78x10⁷, in 2nd day, 4th day, respectively, with reduction percentages 11.5% and 21.2%, respectively, spoiled after 4th day. On the other hand, thyme (1.5%) reduced B. cereus count (cfu/g) from 5.2±1.3 x10⁷ to 4.40±0.58 x10⁷, 3.4±0.17 x10⁷ and 2.4±0.31 x10⁷ at the 2nd day, 4th day and 6th and 8th day, respectively, with reduction percentages 15.4%, 34.6 % and 53.8 %, respectively, spoiled after the 6th day. The results had been recorded verified that nisin (50 ppm) reduced B. cereus count (cfu/g) artificially inoculated into fresh chicken fillet samples from 5.2±1.3 x10⁷ to 3.70± 0.22 x10⁷, 3.0±0.30 x10⁷, 7.60±0.42 x10⁶ and 6.90±0.12 x10⁵ at the 2nd day, 4th day, 6th day and 8th day with reduction percentages 28.8 %, 42.3 %, 85.4 % and 86.7%, respectively and spoiled after 8th day. On the other hand, nisin (100 ppm) reduced B. cereus count (cfu/g) from 5.2±
1.3 × 10^7 to 3.3±1.2 ×10^7, 9.3±0.34 × 10^6, 7.1±0.82 ×10^6, 5.6±0.35 ×10^6 and 3.6±0.11 ×10^6 in 2nd day, 4th day and 6th day, 8th day and 10th day, respectively, with reduction percentages 36.5 %,82.1 %,86.3%,92.2% and 93.1 %, respectively, spoiled after 10th day.

Furthermore, the obtained results indicated that mixture (1.5% and 100 ppm) reduced B. cereus count (cfu/g) artificially inoculated into fresh chicken fillet samples from

5.2±1.3 ×10^7 to 2.5±0.72 ×10^7, 7.2±0.40 ×10^6, 3.7±0.26 ×10^6, 1.1±0.41 ×10^6, 7.8±0.63 ×10^6 and not detected after 2nd day, 4th day and 6th day, 8th day, 10th day and 12th day, respectively, and spoiled after the 12th day, with reduction percentages 51.9%, 86.2 %, 92.9%, 97.9%, 98.5% and 100% , respectively.

### Table 1

<table>
<thead>
<tr>
<th>Days</th>
<th>Control zero</th>
<th>Thyme 1%</th>
<th>Thyme 1.5%</th>
<th>Nisin 50 ppm</th>
<th>Nisin 100 ppm</th>
<th>Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero</td>
<td>5.2±1.7 ×10^7</td>
<td>5.2±1.7 ×10^7</td>
<td>5.2±1.7 ×10^7</td>
<td>3.7±0.22 ×10^7</td>
<td>3.9±0.24 ×10^7</td>
<td>5.2±1.7 ×10^7</td>
</tr>
<tr>
<td>2nd</td>
<td>2.6±0.33 ×10^7</td>
<td>4.6±0.67 ×10^7</td>
<td>4.4±0.58 ×10^7</td>
<td>3.7±0.22 ×10^7</td>
<td>3.9±0.24 ×10^7</td>
<td>2.5±0.72 ×10^7</td>
</tr>
<tr>
<td>4th</td>
<td>SP</td>
<td>4.0±0.78 ×10^7</td>
<td>3.4±0.17 ×10^7</td>
<td>3.0±0.30 ×10^7</td>
<td>9.3±0.34 ×10^7</td>
<td>7.2±0.40 ×10^7</td>
</tr>
<tr>
<td>6th</td>
<td>SP</td>
<td>SP</td>
<td>2.4±0.031 ×10^7</td>
<td>7.6±0.42 ×10^7</td>
<td>7.5±0.26 ×10^7</td>
<td>7.5±0.26 ×10^7</td>
</tr>
<tr>
<td>8th</td>
<td>SP</td>
<td>SP</td>
<td>SP</td>
<td>6.9±0.12 ×10^7</td>
<td>3.6±0.35 ×10^7</td>
<td>1.1±0.41 ×10^7</td>
</tr>
<tr>
<td>10th</td>
<td>SP</td>
<td>SP</td>
<td>SP</td>
<td>SP</td>
<td>3.6±0.11 ×10^7</td>
<td>7.8±0.63 ×10^7</td>
</tr>
<tr>
<td>12th</td>
<td>SP</td>
<td>SP</td>
<td>SP</td>
<td>SP</td>
<td>SP</td>
<td>ND</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Days</th>
<th>Thyme 1%</th>
<th>Thyme 1.5%</th>
<th>Nisin 50 ppm</th>
<th>Nisin 100 ppm</th>
<th>Mixture (1.5% +100 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd</td>
<td>11.5 %</td>
<td>15.4 %</td>
<td>28.8 %</td>
<td>36.5 %</td>
<td>51.9%</td>
</tr>
<tr>
<td>4th</td>
<td>21.2 %</td>
<td>34.6 %</td>
<td>42.3 %</td>
<td>82.1 %</td>
<td>86.2 %</td>
</tr>
<tr>
<td>6th</td>
<td>spoiled</td>
<td>53.8 %</td>
<td>85.4 %</td>
<td>86.3 %</td>
<td>92.9%</td>
</tr>
<tr>
<td>8th</td>
<td>spoiled</td>
<td>spoiled</td>
<td>86.7 %</td>
<td>89.2%</td>
<td>97.8%</td>
</tr>
<tr>
<td>10th</td>
<td>spoiled</td>
<td>spoiled</td>
<td>spoiled</td>
<td>93.1 %</td>
<td>98.5%</td>
</tr>
<tr>
<td>12th</td>
<td>spoiled</td>
<td>spoiled</td>
<td>spoiled</td>
<td>spoiled</td>
<td>100%</td>
</tr>
</tbody>
</table>

### 4. DISCUSSION

_Bacillus cereus_ which frequently associated with food borne diseases (Borge et al., 2001). Everyone is susceptible to _B.cereus_ food poisoning. Some isolates of _B. cereus_ can grow at refrigerated temperature (Valero et al., 2007) and spore can survive at high temperature.

Thyme oil has gained greater acceptance among food technologists due to their better sensory evaluation and antimicrobial properties (Fischer and Phillips, 2006). Nisin inhibits pathogenic food borne bacteria and some Gram-positive food spoilage microorganisms. Nisin can be used alone or in combination with other preservatives or also with several physical treatments (Gharsallaou et al., 2016).

Table (1) illustrated the effects of various concentrations of thyme oil and Nisin on overall acceptability of examined fresh chicken fillet samples. Thyme oil (1%&1.5%) showed overall acceptability till 4th day& 6th day, respectively While nisin (50 ppm,100 ppm) showed overall acceptability till 8th day,10th day respectively. Mixture of (thyme and nisin) showed overall acceptability till 12th day of the experiment.

These results of thyme agreed with Sasse et al. (2009) who reported that many herbs and spices as thyme contain antioxidant components that improve both color and flavor stability in meat.

Also, Salem et al. (2010) indicated that the sensory properties of minced beef samples during cold storage (4°C)were enhanced by treatment by different concentrations of thyme oil (0.5%, 1%, 1.5%) compared to the untreated (control) samples and sample contain 1.5% thyme oil revealed best enhancement of sensory properties as well as the results obtained by Shaltout et al. (2017) revealed that meat samples containing 2% thyme oil demonstrated the highest enhancement of sensory attributes, while the samples treated with 1% of thyme oil demonstrated lower enhancement Mean while these results are not agreed with those obtained by Solomakos et al. (2008) and Giatrakou et al. (2010) who found thymus vulgaris EO on meat was acceptable concerning odor and taste in the range of 0.2 to 0.6 % but unacceptable at 0.9 % on minced beef.

The results recorded in table (2) and table (3) indicated that nisin results agree with those obtained by Nutsuda Sumonsiri (2019) who use nisin (25-75 ppm), the samples treated with 50 and 75 ppm nisin had significantly lower aerobic microbial counts than the control without affecting sensory acceptability. The treated samples also had the significantly higher scores in overall acceptability of the examined fresh chicken breast fillet during cold storage at 4°C.
fillet. Therefore, it is recommended to improve safety of the fresh chicken fillet

5. CONCLUSION

The results of the current study represented that mixture of thyme oil and nisin (1.5% , 100 ppm) improve the quality and sensory characteristics of chicken fillets under chilled storage (4°C) for the economic and public health importance viewpoint.

6. REFERENCES