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

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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 5.2×10^{7}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˚C 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, p-cymene, carvacrol and γ-terpipene. 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 food bio-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 °C).

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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 x 10^7 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* (5.2×10^7 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 slimness 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 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 ×10^7 to 4.60±0.67×10^7 and 4.10±0.78×10^7, 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 ×10^7 to 4.40±0.58 ×10^7, 3.4±0.17 ×10^7 and 2.4± 0.31 ×10^7 at the 2nd day, 4th 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 ×10^7 to 3.70± 0.22 ×10^7, 3.00±1.30 ×10^7, 7.60±0.42 ×10^6 and 6.90 ±0.12 ×10^6 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, 7.10±0.82 ×10^6, 5.60±0.35 ×10^6 and 3.60±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%,89.2% 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 2.50±0.72 ×10^7, 7.20±0.40 ×10^6, 3.70±0.26 ×10^6, 1.10±0.41 ×10^6, 7.80±0.63 ×10^5 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 Effects of various concentrations of thyme and Nisin on over-all acceptability of the examined fresh chicken breast fillet during cold storage at 4˚C.

	Zero day	2nd	4th	6th	8th	10th	12 th
Control+ve	Excellent	Very very poor	SP	SP	SP	SP	SP
Thyme oil							
1%	Excellent	Very very good	Good	Fair	SP	SP	SP
1.5%	Excellent	Very very good	Very very good	Good	Medium	SP	SP
Nisin							
50ppm	Excellent	Very very good	Very good	Good	Fair	SP	SP
100ppm	Excellent	Very very good	Very good	Good	Medium	Fair	SP
Mix							
Mix of Thyme 1.5% and Nisin 100 ppm	Excellent	Very very good	Very very good	Very good	Good	Medium	Fair
Control -ve	Excellent	Medium	Very very poor	SP	SP	SP	SP

SP = spoilage

Table 2 The effects of various concentration of thyme oil and nisin on *B. cereus* (cfu) count of the examined fresh chicken fillet during cold storage at 4˚C.

Day	Control +ve	Thyme .1%	Thyme 1.5%	Nisin 50 ppm	Nisin 100 ppm	Mixture
Zero	5.2±1.3 ×10^7	5.2±1.3 ×10^7	5.2±1.3 ×10^7	5.2±1.3 ×10^7	5.2±1.3 ×10^7	5.2±1.3 ×10^7
2nd	2.60±0.32 ×10^8	4.60±0.67 ×10^7	4.40±0.58 ×10^7	3.70 ±0.22 ×10^7	3.30±1.20 ×10^7	2.50±0.72 ×10^7
4th	SP	4.10±0.78 ×10^7	3.40±0.17 ×10^7	3.00±1.30 ×10^7	9.30±0.34 ×10^6	7.20 ±0.40 ×10^6
6th	SP	SP	2.40±0.031 ×10^7	7.60±0.42 ×10^6	7.10±0.82 ×10^6	3.70±0.26 ×10^6
8th	SP	SP	SP	6.90± 0.12 ×10^6	5.60±0.35 ×10^6	1.10±0.41 ×10^6
10th	SP	SP	SP	SP	3.60±0.11 ×10^6	7.80±0.63 ×10^5
12th	SP	SP	SP	SP	SP	ND

SP = spoilage. **ND =** not detected.

Table 3 Reduction % of *B. cereus* count of the examined fresh chicken fillet samples during cold storage at 4˚C, treated with different concentrations of thyme, nisin and mixture.

Days	Thyme 1%	Thyme 1.5%	Nisin 50 ppm	Nisin 100 ppm	Mixture (1,5% +100 ppm)
2nd	11.5 %	15.4%	28.8 %	36.5 %	51.9%
4th	21.2 %	34.6 %	42.3 %	82.1 %	86.2 %
6th	spoiled	53.8 %	85.4%	86.3%	92.9%
8th	spoiled	spoiled	86.7 %	89.2%	97.8%
10th	spoiled	spoiled	spoiled	93.1%	98.5%
12th	spoiled	spoiled	spoiled	spoiled	100%

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 (Gharsallaoui *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 acceptance than the control sample.

The obtained results were nearly similar to those reported by Ibrahim- Hemmat *et al.* (2014a) who mentioned that nisin had bactericidal effect against *B. cereus* when used at concentrations 20, 40 and 60 ppm. From the other side Roberts and Hoover (1996) found that 10^5 and 10^6 cfu /ml as initial Bacillus spores' concentration were reduced to 10^3 when nisin concentration 1.0 IU/ml was used, while Lee *et al.* (2015) revealed the effect of nisin (100 IU/g and 500 IU/g) on the growth of Bacillus *cereus* inoculated in beef jerky during storage. Generally, mixture of thyme and nisin (1,5% and 100 ppm) proved to be more efficient than other concentrations in suppression of *B. cereus* growth in 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 (4ºC) for the economic and public health importance viewpoint.

6. REFERENCES

1. Borge, Ga; Skeie, M; Sorhaug, T and Langsrud, T; Granum, PE (2001) Growth and toxin profiles of Bacillus cereus isolated from different food sources. Int. J. Food Microbiol., 69 (3) 237- 246.
2. Burt, S. (2004) Essential Oils Their antibacterial properties and potential applications in foods a review. International journal of Food microbiology, 94, 223-253 .
3. Dorman, H.J.D. and Deans, S.G., (2000) Antimicrobial agents from plants antibacterial activity of plant volatile oils. Journal of applied microbiology, 88(2) 308-316
4. Fernandez-Lopez J, Zhi N, Aleson-Carbonell L, Perez-Alvarez J.A., and Kuri V (2005) antioxidant and antibacterial activities of natural extracts application in beef meatballs. Meat sci., 69 371–380 .
5. Fisher, K. and Phillips, C.A. (2006) The effect of lemon, orange and bergamot essential oils and their components on the survival of campylobacter jejuni, Escherichia coli o157 h7, listeria monocytogenes, bacillus cereus and staphylococcus aureus in vitro and in food systems. Journal of applied microbiology, 101 1232–1240 .
6. FDA.(2012) Bacillus cereus. Downloaded from http //www.fda.gov/ Food/ Science Research/Laboratory Methods/Bacteriological Analytical Manual BAM/ ucm 070875.
7. Gharsallaoui, A.; Oulahal N.; Joly, C. and Degraeve, P. (2016) Nisin as a food preservative part 1 physicochemical properties, antimicrobial activity, and main uses. Crit Rev Food Sci Nutr., 56(8) 1262-1274 .
8. Gibbons, S. (2008) phytochemicals for bacterial resistance strengths, weaknesses and opportunities. Planta Medica, 74, 594- 602.
9. Giatrakou, V., Ntzimani, A. and Savvaidis, I.N., (2010) Combined chitosan-thyme treatments with modified atmosphere packaging on a ready-to-cook poultry product. Journal of food protection 73, 663-669.
10. Hassan, M. A. (1999). Follow up of some pathogens in meat products and their resistance to certain preservatives. Beni-Suef Vet. Med. J, 9(3), 417-429.
11. Ian, M.G.; Steven, R.B. and Wilfred A.D. (2011) Mechanism of inhibition of bacillus cereus and bacillus anthracis spore outgrowth by the lantibiotic nisin. J. Chem. Biol., 6 744-752 .
12. Ibrahim H.M.; Salm, A.M.; Khater, D.F. and Ghanyem, H. R. (2014) Antimicrobial effect of some preservatives on bacillus cereus isolated from some meat products. Benha Veterinary Medical Journal. 26 (1) 75-83.
13. International Organization for Standardization“ ISO” (2006) Downloaded from https //www.iso.org. International Organization for Standardization. No21871. Microbiology of food and animal feeding stuffs – Horizontal methods for detection of bacillus cereus.
14. .Koblitz , M.G.B.(2011) Raw materials for food composition and quality control.Rio de Janeiro (RJ, Brazil) Guanabara Koogan; .p. 320.
15. Lee N.; Kim H.W.; Yeon L.J.; Ahn D.U.; Cheon-Jei Kim and Hyun-Dong Paik (2015) Antimicrobial effect of nisin against bacillus cereus in beef jerky during storage. Korean j. Food sci. An. 35(2) 272-276 .
16. Mahmoud, S.S. and Croteau, R.B., (2002) Strategies for transgenic manipulation of monoterpene biosynthesis in plants. Trends in Plant Science, 7(8) 366-373.
17. Mandal, S., and DebMandal, M. (2016). Thyme (Thymus vulgaris L.) oils. In Essential oils in food preservation, flavor and safety (825-834). Academic .
18. Papagianni, M. and Anastasiadou, S. (2009) Pediocins The bacteriocins of Pediococci. Sources, production, properties and applications. Review. Microbial Cell Factories, 8 1-16 .
19. Pearson, A. M. and Tauber, F.W. (1984) Processed meat. 2nd AVI Publishing Company, Inc .
20. Rahimi, E., Abdos, F., Momtaz, H., Torki Baghbadorani, Z., and Jalali, M. (2013). Bacillus cereus in infant foods prevalence study and distribution of enterotoxigenic virulence factors in Isfahan Province, Iran. The Scientific World Journal.
21. Roberts, C. M., and Hoover, D. G. (1996). Sensitivity of Bacillus coagulans spores to combinations of high hydrostatic pressure, heat, acidity and nisin. Journal of Applied Bacteriology, 81(4), 363-368.
22. Salem, A.M.; Amine, R.A. and Gehan, S. (2010) Studies on Antimicrobial and antioxidant efficiency of some essential oils in minced beef. Journal of American Science, 6 (12) 691-700.
23. Sasse A, Colindres P and Brewer M.S (2009) Effect of natural and synthetic antioxidants on oxidative stability of cooked, frozen pork patties. J Food Sci. 74 30-35.
24. Shaltout, F. A., and Koura, H. A. (2017). Impact of some essential oils on the quality aspect and shelf life of meat. Benha Veterinary Medical Journal, 33(2), 351-364.
25. Solomakos, N., Govaris, A., Koidis, P., and Botsoglou, N. (2008) The antimicrobial effect of thyme essential oil, nisin, and their combination against listeria monocytogenes in minced beef during refrigerated storage. Food microbiology 25, 120-127.
26. Slabyj, B., Bushway, A., and Hazen, R. (2003). Microbiological quality and safety of food. University of Maine Orono, ME, 4473.
27. Sumonsiri, N. (2019). Effect of nisin on microbial, physical, and sensory qualities of micro-filtered coconut water (Cocos nucifera L.) during refrigerated storage. Current Research in Nutrition and Food Science, 7(1), 236. .
28. Valero, M., Hernandez-Herrero, L.A. and Giner, M.J. (2007) Survival, isolation and characterization of a psychrotrophic bacillus cereus strain from a mayonnaise-based ready-to-eat vegetable salad. Food microbiology, 24 671-677 .
29. Wilkinson, J.M., Hipwell, M., Ryan, T., and Cavanagh, H.M.A. (2003) Bioactivity of backhousia citriodora antibacterial and antifungal activity. J. Agric. Food Chemi., 51 76–81.
30. Zaki, Eman, M.S. and Shehata, Amal, A. (2008) Incidence of some enterotoxigenic food poisoning microorganisms in chicken meat products. Giza Vet. Med. J.; 56(3) 255-266.