Annals of Clinical and Medical Case Reports

Research Article ISSN 2639-8109 |Volume 12

The Synergistic Effect of Two Different Types of Natural Additives on Bacillus Cereus in Chicken Burger

Edris AM[1], Haraz SM[2], Arab WS[1] and Radwa AL[3*]

[1]Department of Food Hygiene and control, Faculty of Veterinary Medicine, Benha University, Egypt

[2]Veterinary doctor in directorate Gabria government, Egypt

[3]Department of Food Hygiene, Animal Health Research Institute, Tanta, Egypt. Agricultural Research Center, Egypt

[*]Corresponding author:

Radwa A Lela,
Department of Food Hygiene, Animal Health Research Institute, Tanta, Egypt. Agricultural Research Center, Egypt

Received: 09 Nov 2023
Accepted: 05 Dec 2023
Published: 13 Dec 2023
J Short Name: ACMCR

Citation:

Radwa AL, The Synergistic Effect of Two Different Types of Natural Additives on Bacillus Cereus in Chicken Burger. Ann Clin Med Case Rep. 2023; V12(3): 1-6

Keywords:

Nisin; Thyme oil; Bacillus cereus; Shelf life of chicken burger

1. Abstract

Use of chemical preservatives is recently being considered by customers due to concerns related to negative health. Natural preservatives are very vital for enhancing food safety and shelf life. They are safe because they can limit microbial resistance and meet consumers' demands for healthier foods. There are types of natural preservatives. Plant-derived as Thyme and microbial metabolites as Nisin. Thyme has much attention due to its high content phenolic compounds, antimicrobial, antioxidant properties also influence food sensory properties including the flavor, taste, color, texture, and acceptability of food and it is reasonably priced and available for use. Nisin is bacitracin that has got FDA approval for application as a food preservative. But it is very expensive and hardly commercially available. The current study aimed to study the effect of thyme, nisin, and their combination on Bacillus cereus inoculated in chicken burger. Found that combination of two different types of preservatives (thyme and Nisin) have synergistic effect as antimicrobial and enhance food sensory properties more than thyme or nisin alone in different concentration. The results showed that overall acceptability of the combination is to 14 th day of storage. In contrast, control positive and control negative showed overall acceptability till 4th, 6th day of storage respectively.

2. Introduction

Chickens are the most ubiquitous of all livestock species, and are found more or less everywhere inhabited by people. To enhance human growth and health, chicken meat provides a considerable part of microelements like as copper, iron, zinc, calcium, phosphorus, and cobalt. Besides, vitamins such as vitamin B group can also be provided when meat chicken is consume [5]. Because they are nutritious, delicious, and affordable, chicken meat and meat products such chicken burgers are important sources of protein, energy, vitamins, and minerals around the world [10]. The microbial safety of foods continues to be a major concern to consumers, food industries throughout the world. Many food preservation strategies have been used traditionally for the control of microbial spoilage in foods but the contamination of food and spoilage by microorganisms is a problem yet to be controlled adequately. Although synthetic antimicrobials are approved in many countries, the recent trend has been for use of natural preservatives, which are alternative sources of safe, effective and acceptable natural preservatives. Plants contain innumerable constituents and are valuable sources of new and biologically active molecules possessing antimicrobial properties [12].

Bacillus cereus is one of the food-borne disease causing bacteria. Bacillus Species and related genera have long been troublesome to food producers on account of their resistant endospores. Bacillus cereus is well known as a cause of food poisoning, and much more is known about the toxins produced by various strains of this species [2].

Bacillus cereus produces two types of toxins – emetic (vomiting) and diarrheal causing two types of illness. The emetic syndrome is occured by emetic toxin produced by the bacteria during the growth phase in the food. The diarrhoeal syndrome is happened by diarrheal toxins produced during growth of the bacteria in the small intestine [4].

Thyme oil has an excellent antibacterial activity against important food pathogens such as Bacillus cereus. The bacteriostatic and bactericidal activities of thyme is higher against the Gram-positive pathogens than the Gram-negative ones. It was also active in counteracting the biofilm formation by Bacillus cereus [16].

Nisin is a preservative and antibacterial agent that is used to inhibit the germination and outgrowth of spores; it alters cell properties in bacteria to render it harmless. Nisin is currently recognized in approximately 50 countries as a safe food preservative. Nisin is anti-microbial agent, which is active against Gram positive bacteria including spore formers, but does not inhibit the majority of Gram-negative bacteria. It is also stable under refrigerated conditions [14].

The aim of the study is to throw alight on the effect of thyme and nisin alone and their mixture on B.cereus in chicken burger.

3. Materials and Methods

3.1. Preparations of Inoculate

Bacillus cereus strain was obtained from Animal Health Research Institute (AHRI), Dokki, with recommended dose (5.2 x 107 CFU/ml) as recorded by McFarland's nephelometer standards according to Slabyj et al., [19].

3.2. Thyme Preparation

Thyme oil provided by (ARC) Agriculture Research Center, Egypt '' by hydro-distillation method, Tween 80 added to essential oils before applying in samples as diluent and therefore, easily distribution and dissolving According to Wilkinson et al., [21].

3.3. Nisin preparation was prepared at concentrations 50 and 100 ppm according to Hassan [8].

3.4. Preparation of Chicken Burger Samples

A total amount of 2100 gm of raw chicken burger was been purchased from a butcher shop from Tanta city, it taken and transferred directly to the laboratory under complete aseptic conditions without undue delay. The sample was divided to 7 equal groups (3 x100 gm for each) first group was control negative (no treatment), second group used as control positive, third group and fourth groups were treated with Thyme oil (1%-1.5%), respectively. Fifth and sixth groups were treated with Nisin (50 ppm- 100 ppm), respectively, while the seventh groups treated with mixture of both (thyme 1.5% and Nisin 100 ppm).

All the groups were packed in a separate sterile polyethylene bags and stored in domestic refrigerator at nearly ±40c, each sample was analyzed at zero, 2 nd, 4th, 6th, 8th, 10th and 12th days during storage for presence of bacillus cereus and sensory properties. This work was been conducted in triplicate.

Sensory examination: according to Pearson and Tauber [15].

Bacteriological analyses: on Mannitol Egg Yolk Polymyxin (M.Y.P) media according to [20].

3.5. Statistical Analysis

The obtained results were statistically evaluated by application of one-way ANOVA test according to [6].

4. Results

Table 1, illustrated the effects of various concentrations of thyme, nisin and mixture of them on overall acceptability of groups. Thyme (1% & 1.5%) showed accepted sensory properties extended to 6^{th}, 8^{th} day of storage respectively. Nisin (50 ppm & 100 ppm) showed overall acceptability till 10^{th}day, 12^{th} day of storage respectively while mixture (thyme 1% ,nisin 100 ppm) showed overall acceptability to 14^{th} day of storage. In contrast, control positive and control negative showed overall acceptability till 4^{th}, 6^{th} day of storage respectively.

Table 2&3 and Figure 1, illustrated the antimicrobial effects and reduction percentage of various concentrations of thyme, nisin and mixture of them on counts of *B. cereus* artificially inoculated into chicken burger. Thyme oil (1% &1.5%) decreased count of *B.cereus* (cfu/g) from $5.2x10^7$ (initial load) to $3.4x10^7$and $8.5\ x10^6$ with reduction percentages 34% and 84% on 6^{th}day and 8^{th} day of storage, respectively. Nisin (50 ppm and100 ppm) decreased count of *B. cereus* (cfu/g) to $1.6\ x10^6$ and $2.4x10^5$ with reduction percentages 96.5% and 100 % on 10^{th} day and 12^{th} day of storage, respectively. Mixture of (thyme and nisin) decreased count of *B. cereus* (cfu/g) to $5.3\ x\ 10^5$ with reduction percentage100 % on 10^{th}, 12^{th} and 14^{th} day of storage. In control positive group *B. cereus* count increased from $5.2x10^7$ (initial load) to $3.6x10^8$ on 4^{th} day so, the differences between the effects of various concentrations of thyme and nisin and their mixture on counts of *B.Cereus* (cfu/g) artificially inoculated into chicken burger sample were significantly different.

Table 1: over all acceptability (sensory evaluation) of the examined chicken burger samples during cold storage at 40c.

Groups	Zero day	2nd	4th	6th	8th	10th	12th	14th
Control -	Excellent	very very good	Medium	Fair				
Control +	Excellent	Medium	Fair	SP	SP	SP	SP	SP
Thyme oil								
1%	Excellent	very very good	Good	Medium	SP	SP	SP	SP
1.50%	Excellent	very very good	very very good	Good	Medium	SP	SP	SP
Nisin								
50ppm	Excellent	very very good	Very good	Good	Medium	Fair	SP	
100ppm	Excellent	very very good	Very good	Good	Good	Medium	Fair	
Mix	Excellent	very very good	very very good	Very good	Good	Good	Medium	Fair

Sp: spoiled

Table 2: The effects of various concentration of thyme oil and nisin on counts of B.cerus (cfu)

	Control +	**Thyme 1%**	**Thyme 1.5%**	**Nisin 50 ppm**	**Nisin 100 ppm**	**Mixture**
Zero	$5.2x10^{7}\pm$ $0.84\ x10^{7}$	$5.2x10^{7}\pm$ $0.84\ x10^{7}$	$5.2x10^{7}\pm$ $0.84\ x10^{7}$	$5.2x10^{7}\pm$ $0.84\ x10^{7}$	$5.2\times10^{7}\pm$ 0.84×10^{7}	$5.2\times10^{7}\pm$ 0.84×10^{7}
2^{nd}	$7.3x10^{7}\pm$ $0.52\ x10^{7}$	$4.2x10^{7}\pm$ $0.63X10^{7}$	$3.8x10^{7}\pm$ $0.60\ X10^{7}$	$3.5\times10^{7}\pm$ 0.51×10^{7}	$2.7\times10^{7}\pm$ 0.14×10^{7}	$2.4\times10^{7}\pm$ 0.32×10^{7}
4th	$3.6x10^{8}\pm$ $0.12\ x10^{8}$	$4.0x10^{7}\pm$ $0.43\ X10^{7}$	$2.7x10^{7}\pm$ $0.20X10^{7}$	$2.4\times10^{7}\pm$ 0.87×10^{7}	$8.5\times10^{6}\pm$ 0.23×10^{6}	$6.4\times10^{6}\pm$ 0.47×10^{6}
6th	SP	$3.4x10^{7}\pm$ $0.32X10^{7}$	$1.4x10^{7}\pm$ $0.33X10^{7}$	$7.7\times10^{6}\pm$ 0.73×10^{6}	$4.7\times10^{6}\pm$ 0.50×10^{6}	$1.8\times10^{6}\pm$ 0.47×10^{6}
8th	SP	SP	$8.5\times10^{6}\pm$ 0.18×10^{6}	$3.5\times10^{6}\pm$ 0.45×10^{6}	$7.5\times10^{5}\pm$ 0.37×10^{5}	$5.3\times10^{5}\pm$ 0.11×10^{5}
10th	SP	SP	SP	$1.6\times10^{6}\pm$ 0.19×10^{6}	$2.4\times10^{5}\pm$ 0.18×10^{5}	ND
12th	SP	SP	SP	SP	ND	ND
14th	SP	SP	SP	SP	SP	ND

ND: not detected

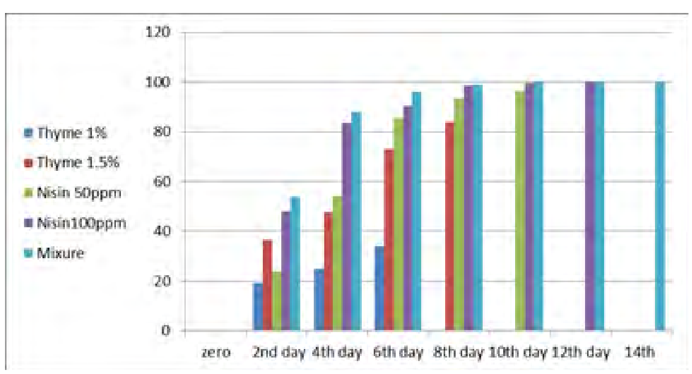

Figure 1: Reduction % in count of B. cereus count

Table 3: Reduction % in count of B.cereus count.

Mixture (1,5% +100 ppm)	Nisin 100 ppm	Nisin 50 ppm	Thyme 1,5%	Thyme 1%	Days
53.80%	48.10%	23.60%	26,3%	19.20%	2nd
88%	83.60%	54%	47.60%	25%	4th
96%	90.10%	85,3%	73.10%	34%	6th
99%	98.50%	93.30%	84%	spoiled	8th
100%	99.50%	96.50%	Spoiled	spoiled	10th
100%	100%	spoiled	Spoiled	spoiled	12th
100%	spoiled	spoiled	spoiled	spoiled	14th

5. Discussion

Symptoms of *B. cereus* foodborne toxicoinfection depend on the type of the toxin produced as there are diarrheal or emetic forms. The diarrheal form is characterized by the watery diarrhea and abdominal cramps with an incubation period of 6-15 hrs. While the emetic form is characterized by vomiting, and nausea with an incubation period of 30 minutes to 6 h [3].

In table 1, revealed that the all groups have excellent score at zero day of storage, while in 4th day the control positive group showed signs of deterioration with bad odor and texture. At 6th day the control negative group decomposed, while the other treated groups have very good to good overall acceptability but treated with thyme 1.5% and nisin 100 ppm were very good acceptability. At 6th day the control negative group decomposed, while thyme 1.5%, nisin 50ppm, 100 ppm had good over all acceptability while mixture (1.5 %, 100 ppm) had very good score of over-all Acceptability. at 8th day thyme oil was decomposed while treated with thyme oil 1.5 ppm were medium, nisin 50 ppm was fair but mixture was good overall acceptability. At 10th day of treatment thyme1.5%, nisin 50 ppm were decomposed, while nisin 100 ppm was medium but mixture was good overall acceptability. At last the 12th day the 100 ppm group was decomposed while mixture treated samples was fair acceptability.

These results agreed with those obtained by [1]. There was a decline of acceptability began after the first day of storage with marked reduction of odor, color, texture and overall acceptability values in the control samples at the 4th day of storage.

These results Also agreed with [17], who reported that many herbs and spices as thyme contain antioxidant components that improve both color and flavor stability in meat.

The results were in line with the [23] who found that essential oils enhanced the organoleptic character of chicken meat. The sample containing thyme had a better sensory appearance than the control sample.

The results of nisin agree with those obtained by [8], who used the treated and control have excellent score at zero day and first day of treatment while in second day the treated and control were very very good with starting the decrease the quality of texture and odor of control .meanwhile the third day the control score start to decrease with medium score while 30 ppm,50 ppm had very good score of over-all acceptability at last the fifth day the 10ppm group was decomposed while 30ppm treated samples was fair over all acceptability otherwise 50ppm was good to medium.

This results of nisin Also agree with those obtained by [13] 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 results recorded in Table 2 and 3, indicated that thyme (1%) reduced ***B. cereus*** count (cfu/g) artificially inoculated into chicken Burger samples from 5.2×10^7± 0.84 x$10^7$to 4.2×10^7± 0.63 ×10^7, 4 ×10^7± 0.43 × 10^7 ,3.4 x10^7±0.32 x10^7 in 2nd day, 4th, 6th day respectively, with reduction percentages 19.2% and 25% ,34% respectively, spoiled after 6 th day. Meanwhile thyme (1.5%) reduced B. cereus count (cfu/g) artificially inoculated into chicken Burger samples from 5.2×10^7± 0.84 x10^7 to 3.8 x10^7± 0.60 ×10^7, 2.7×10^7± 0.20 ×10^7,1.4 x10^7±0.33 x$10^7$and 8.5×10^6± 0.18 ×10^6 in 2nd day, 4th day 6th day and 8th day respectively, with reduction percentages 26.3%, 47.6% and 73.1% ,84% in 2nd day, 4th day 6th day and 8th day respectively, spoiled after 8th day.

The results recorded Table 2 and 3, indicated that nisin (50 ppm) reduced ***B. cereus*** count (cfu/g) artificially inoculated into chicken Burger samples from 5.2×10^7± 0.84 x10^7 to 3.5×10^7± 0.51×10^7, 2.4 ×10^7± 0.87×10^7,7.7 x10^6± 0.73 x10^6,3.5x10^6±0.45 x10^6 in 2nd day, 4th day ,6th day, 8th and 10th day respectively, with reduction percentages 23.6%,54%,85.3% ,93.3% and 96.5 % respectively and spoiled after10th day. Meanwhile nisin (100 ppm) reduced ***B.cereus*** count (cfu/g) artificially inoculated into chicken Burger samples from 5.2×10^7± 0.84 x10^7 to 2.7 x10^7± 0.14 x10^7, 8.5 x10^6± 0.23 x10^6 , 4.7 x10^6±0.50 x10^6, 7.5 x105±.37x10^5,2.4x10^5±0.18x10^5 in 2nd day, 4th day and 6th day ,8th day and 10th day, respectively, with reduction percentages 48.1%,83.6 % ,90.1 %,98.5%, 99.5% and 100% respectively, spoiled after 12th day.

The results recorded Table 1 and 2, indicated that mixture (1.5%,100 ppm) reduced ***B.cereus*** count (cfu/g) artificially inoculated into chicken Burger samples from 4.9×5.2×10^7± 0.84 x10^7,-to 2.4 x10^7±0.32 x10^7, 6.4 x10^6±0.47 x10^6, 1.8 x10^6±0.40x10^6,5.3 x10^5± 0.1 1 x10^5, ND after 2nd day , 4th day and 6th day ,8th day and 10th day, 12th till 14th day respectively, spoiled after 14th day, with reduction percentages 53.8%,88% ,96%,99%,98.5% and 100% respectively .

The observed reduction in microbial counts can be attributed to the inhibitory effect of thyme on spoilage bacteria [7]. However, samples were considered spoiled if total bacterial counts were above 7 log CFU/g, In the current study chicken samples treated with thyme did not exceed the value of 7.0 log CFU/g for TBC, which was considered as the upper acceptability limit for fresh meat [18] till the 12th day of storage.

The obtained result of nisin 50 ppm were nearly similar to who [8] reported that Nisin at (50 ppm) reduced ***B. cereus*** count (cfu/g) artificially inoculated into minced meat samples from $5.0\times10^7\pm 0.82\times10^7$ to $9.74\times106\pm 2.03\times10^6$,$7.36\times10^5\pm 1.15\times10^5$, ND and ND after 1st day, 2nd day, 3rd day, 4th day and 5th day, respectively, with reduction percentages 80.5%, 98.5%, 99.9%, 100% and 100%, respectively.

The obtained result of nisin were nearly similar to [9] reported that ***B.cereus*** more than the use of Nisin 100g/ton alone. The use of Nisin 300g/ton decreased the count of ***B.cereus*** to 2.68 log cfu /g, the use of Nisin 200g/ton decrease the count of ***B.cereus*** to 2.86 log cfu /g, the use of Nisin 100 g/ton decreased the count of ***B.cereus*** to 3.22 log cfu /g and also said that the addition of 3 concentrations of nisin (100, 200 and 300 g/ton) reduced log the count of inoculated ***B. cereus*** by 3-5 log cycles; similar results were recorded by **Roberts and Hoover (1996)** found that ***B.cereus*** initial count was reduced by three log cycles when Nisin concentration was 1.0 I.U. / ml.

Nisin also inhibits the outgrowth of germinated bacterial spores, Organisms from several families of bacteria, including the Bacilli , form small endospores in nutrient-deprived conditions, allowing survival over extended periods of time, which would not be possible as vegetative cells [11].

The recent study revealed the best concentration of Thyme And nisin controlling ***B.cereus*** is (1.5% - 100 ppm) reduced ***B. cereus*** count (cfu/g) artificially inoculated into chicken burger samples with reduction percentages reached to100% with sensory characteristics very good score of over-all acceptability over all the experimental time Therefore, It is recommended to improve safety of the chicken burger.

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 burger under chilled storage (4^{o}C) for the economic and public health importance viewpoint.

References

1. Salem AM, Mohamed EF, Selim EM. Antimicrobial effect of some natural oils on Bacillus cereus in minced beef. 2018; 34(2): 149-56.
2. Anita T, Swaid A. Bacillus cereus food poisoning International and Indian perspective. J Food Sci Technol. 2014; 52(5): 2500-11.
3. Darwish WS, El-Ghareeb WR, Alsayeqh AF, Morshdy AEM. Foodborne intoxications and toxicoinfections in the Middle East. In Food Safety in the Middle East. Academic Press. 2022; 109-41.
4. Ehling-Schulz M, Fricker M, Grallert H, Rieck P, Wagner M, Scherer S. Cereulide synthetase gene cluster from emetic Bacillus cereus: Structure and location on a mega virulence plasmid related to bacillus anthracis toxin plasmid pXO1. BMC Microbiol. 2006; 6: 20.
5. El Bayomi RM, Darwish WS, Elshahat SS, Hafez AE. Human health risk assessment of heavy metals and trace elements residues in poultry meat retailed in Sharkia Governorate, Egypt. Slovenian Veterinary Research. 2018; 55(20): 211-9.
6. Feldman D, Hoffman R, Simpson J. The solution for data analysis and presentation graphics.2nd Ed. Abacus Land cripts, Inc., Barkeley, CA, USA. 2003.
7. Gutierrez J, Barry-Ryan C, Bourke P. Antimicrobial activity of plant essential oils using food model media: efficacy, synergistic potential and interactions with food components. Food microbiology. 2009; 26(2): 142-50.
8. Hassan MA. Follow up of some pathogens in meat products and their resistance to certain preservatives. Benisuef Vet. Med. J. 1999; 9(3): 417-29.
9. Mohamed WS, Ghanyem HR. Effect of some preservatives on Bacillus cereus isolated from some meat products. Assiut Veterinary Medical Journal. 2015; 61(146): 1-7.
10. Morshdy AEM, Al-Mogbel MS, Mohamed ME, Elabbasy MT, Elshafee AK, Hussein MA. Bioactivity of essential oils for mitigation of Listeria monocytogenes isolated from fresh retail chicken meat. Foods. 2021a; 10: 3006.
11. Montville TJ, DeSiano T, Nock A, Padhi S, Wade D. Inhibition of Bacillus anthracis and potential surrogate bacilli growth from spore inoculated by nisin and other antimicrobial peptides. J. Food Prot. 2006; 69: 2529-33.
12. Negi SP. Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application. J. 2012; 156(1): 7-17.
13. Sumonsiri N. Effect of Nisin on Microbial, Physical, and Sensory Qualities of Micro-filtered Coconut Water (Cocos nucifera L.) during Refrigerated Storage. nutrition and food science. 2019.
14. Patricia C, Mauricio R, Marta S, Ana M. Combined effect of water activity and pH on the inhibition of Escherichia coli by Nisin. J. Food Protec. 2001; 64(10): 1510-4.
15. Pearson AM, Tauber FW. Processed meat. 2ndAVI Publishing Company, Inc. 1984.
16. Sateriale D, Forgione G, Anna De Cristofaro G, Pagliuca C, Colicchio R, Salvatore P, et al. Antibacterial and Antibiofilm Efficacy of Thyme (Thymus vulgaris L.) Essential Oil against Foodborne Illness Pathogens, Salmonella enterica subsp. enterica Serovar Typhimurium and Bacillus cereus. Antibiotics. 2023; 12(3): 485.
17. Sasse A, Colindres P, Brewer Ms. Effect of natural and synthetic antioxidants on oxidative stability of cooked, frozen pork patties. J Food Sci. 2009; 74: 30-5.

18. Senter SD, Arnold JW, Chew V. APC Values and Volatile Compounds Formed in Commercially Processed, Raw Chicken Parts During Storage at 4 and 13 C and Under Simulated Temperature Abuse Conditions. Journal of the science of food and agriculture. 2000; 80(10): 1559-64.

19. Slabyj B, Bushway A, Hazen R. Microbiological quality and safety of food.University of Maine Orono, ME. 2003; 04473.

20. Tallent SM, Kotewicz KM, Strain EA, Bennett RW. Efficient isolation and identification of bacillus cereus group. J. Aoac int. 2012; 95(2): 446-51.

21. Wilkinson JM, Hipwell M, Ryan T, Cavanagh HMA. Bioactivity of backhousia citriodora: antibacterial and antifungal activity.j.agric. food chemi. 2003; 51: 76-81.

22. Massilia RM, Melgar JM, Fortuny RS, Belloso OM. Control of pathogenic and spoilage microorganisms in fresh-cut fruits and fruit juices by traditional and alternative natural antimicrobials. Compr. Rev. Food Sci. Food Saf. 2009; 8(3): 157-80.

23. Zhang H, Wu J, Guo X. Effects of antimicrobial and antioxidant activities of spice extracts on raw chicken meat quality Food Science and Human Wellness. 2016; 5: 39-48.