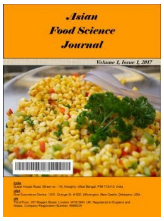

Asian Food Science Journal

3(1): 1-9, 2018; Article no.AFSJ.41802

Decontaminating Effect of Organic Acids and Natural Compounds on Broiler Chicken Meat Contaminated with *Salmonella typhimurium*

D. N. N. Madushanka[1], T. S. P. Jayaweera[1], J. M. C. S. Jayasinghe[1], D. G. Yasawathie[1] and H. A. D. Ruwandeepika[1*]

[1]Department of Livestock Production, Faculty of Agriculture Sciences, Sabaragamuwa University of Sri Lanka, Belihuloya, Sri Lanka.

Authors' contributions

This work was carried out in collaboration between all authors. Authors DNNM, TSPJ and HADR designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Aurhors JMCSJ, DNNM, HADR and DGY managed the analyses of the study. Authors DNNM and HADR managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AFSJ/2018/41802
Editor(s):
(1) Vodnar, Dan – Cristian, Assistant Professor, Department of Food Biotechnology, Food Chemistry and Food Microbiology, University of Agricultural Sciences and Veterinary Medicine, Cluj Napoca, Romania.
Reviewers:
(1) Slim Smaoui, Tunisia.
(2) K. Immaculate Jeyasanta Suganthi Devadason, Marine Research Institute, India.
Complete Peer review History: http://prh.sdiarticle3.com/review-history/25165

Original Research Article

Received 25th March 2018
Accepted 5th June 2018
Published 18th June 2018

ABSTRACT

Aims: The study was carried out to investigate the effect of natural compounds and organic acids on *Salmonella typhimurium* in broiler chicken meat.
Study Design: This is a laboratory-controlled experimental design.
Place and Duration of Study: Department of Livestock Production, Sabaragamuwa University of Sri Lanka, between February 2014 and April 2014.
Methodology: Approximately equal sized (4x4x1.5 cm), shaped (cuboidal) and same weight 10g±0.2) chicken meat samples were taken, sterilized using UV and contaminated with *Salmonella typhimurium* (ATCC 14028) at two cell densities (10^2 and 10^8 Colony Forming Units (CFU)/g of meat). Subsequently, meat samples were treated with 1% solutions of both Chemical (Citric, Acetic and Lactic) and Natural compounds (Cardamom, Nutmeg and Mace) for 30 seconds. Treated

**Corresponding author: E-mail: ruwandeepika@yahoo.co.uk, druwandeepika@gmail.com;*

samples were ground and serially diluted for the enumeration of recovered bacteria. Data were analyzed using SAS followed by a Tukey's post-hoc test at 95% significance level.
Results: Lactic, Acetic and Citric acids have shown 66% (25 CFU/g), 55% (33.33 CFU/g) and 51% (36.6 CFU/g) reduction of colony count at 10^2 CFU/g inoculation and 88% (8.13×10^6 CFU/g), 87% (9.3×10^6 CFU/g) and 72% (2.03×10^7 CFU/g) reduction at 10^8 CFU/g inoculation level. Treatment with Nutmeg, Mace and Cardamom showed 85% (10 CFU/g), 83% (11.6 CFU/g) and 66% (23.3 CFU/g) reduction at 10^2 CFU/g and 89% (7.3×10^6 CFU/g), 89% (7.5×10^6 CFU/g) and 72% (1.96×10^7 CFU/g) reduction at 10^8 CFU/g inoculation level. Natural compounds showed a 20% greater reduction of colony count in broiler chicken compared to chemical compounds.
Conclusion: It is concluded that the natural compounds and weak organic acids have an anti-*S. typhimurium* effect, hence it can be used for decontamination process of poultry meat.

Keywords: Chicken meat; natural compounds; organic acids; Salmonella.

1. INTRODUCTION

The meat industry is one of the world's biggest industries which has shown a rapid growth over the past decades. Chicken consumption appears to be the best solution to battle against malnutrition problems such as Protein Energy Malnutrition throughout the world. It is estimated that more than 2 billion people on the globe lack key nutritional supplements such as vitamin A, Iodine, Iron and Zinc [1] and generally the chicken meat is comparatively cheap and has fewer health risks [2]. World's meat manufacture is expected to flourish slightly in 2015 to achieve 415 million tons, an increase of 6 million tons or 1.4% in comparison to 2012. In 2011, the per capita consumption of chicken among Sri Lankans has increased to 5.7 kg in comparison to 5 kg in 2010 [1].

Even though meat plays a vital role in our day to day life, it still comes with some penalties. As far as the meat consumption is concerned it said to be, more you eat meat, more you were in the trouble [2]. Coronary heart disease and Foodborne illnesses are two of major risk concerns that have direct links to the meat consumption. Foodborne illnesses include a wide variety of illnesses and are a growing public health concerns globally. They are caused by consumption of foods infected with harmful bacteria or substances [3,4].

The contamination of meals may occur at any stage in the process from meals production to consumption ("farm to fork") and can outcome from polluting the environment, including contamination of water, ground or air. The most common medical demonstration of foodborne illness requires the way of digestive symptoms; however, such illnesses can also have nervous, gynecological, immunological and other signs. Multi organ failing and even melanoma may outcome from the consumption of infected foods, thus comprising a significant pressure of impairment as well as death rate [5,6].

Salmonella is a typical foodborne pathogen that has triggered infrequent sickness and outbreaks for over 100 decades. *Salmonella typhimurium* was the most common serotype of *Salmonella* revealed by the Center for Disease Control and Prevention [7]. As a result of increasing demand of chicken consumption, synthetic antibiotics have been used in large quantities to deal with pathogenic microorganisms in chicken production industries which could be a threat to human life. Even though the synthetic antibiotics are much effective, frequent use of them has also triggered drug resistance among harmful pathogenic organisms. In recent years, there were many reported cases all around the world about drug resistance to human pathogenic microorganisms [8]. Moreover, it has become an alarming concern as microorganisms show the ability to resist multiple drugs. This scenario has prompted scientists to conduct research and find alternatives to common antibiotics.

Since ancient times, plants have been utilized as an important source of medicines as they are a reservoir of chemical agents with antimicrobial properties [9].

Chemicals usually destroy harmful bacteria by harmful or toxic responses and efficient disinfectants are often harmful to individual and human cells as well. Natural compounds such as Nutmeg have shown antibacterial effect and have been used in food industries as a food preservatives [10]. Also some weak acids such as acetic acids and lactic acid have been used in food industries as a food preservatives [11]. Therefore the present study was carried out to investigate the effects of natural compounds (Mace, Nutmeg and cardamom) and weak acids (acetic, lactic and citric acids) on *Salmonella typhimurium* in chicken meat.

2. MATERIALS AND METHODS

2.1 Preparation of Chicken Meat

Frozen chicken meat was used for the preparation of sample in the study. Meat samples were cut into approximately equal size 4x4x1.5 cm shaped (cuboidal) pieces and same weight 10±0.2 g to make sure the uniformity of the samples.

2.2 Preparation of Organic Acids

Three organic acids were used in the study (Acetic, Citric and Lactic acids). One percent solutions of acids were prepared by mixing them with appropriate amounts of sterilized distilled water according to the studies done by [12,13,14]. All the acids that were used as treatments had lower pH levels/ Acidic pH levels. At the 25°C and 1 atm pressure, Lactic, Acetic and Citric acids had following pH values 3.51, 3.91 and 4.18.

2.3 Preparation of Natural Compounds

In order to detect the effect of natural compounds on bacterial growth, Nutmeg, Mace and Cardamom were selected (Fig. 1). The fresh nutmeg, mace and cardamom were obtained from the local market and were cleaned, washed in sterile distilled water and oven dry. Surface of the natural products was sterilized using UV in a sterile laminar flow chamber, and they were grinded to get the powder aseptically using a grinder. The concentration, 1% was made by dissolving the grinded compounds with appropriate volumes of sterilized distilled water and sterilized by filter sterilization.

2.4 Preparation of Bacterial Culture

Salmonella typhimurium (ATCC 14028) (kindly donated by Prof Indrani Karunasagar, Dean, Faculty of Biomedical Sciences, Nitte University, Mangalore, India) was used to contaminate meat samples artificially. Culture was grown in Luria Bertani broth and incubated at 37^0C for overnight. Cells were harvested and washed by centrifugation and number of cells was determined according to the optical density (at 640nm) by using spectrophotometer (Thermo scientific, Genesys 10S UV-V1S) according to McFarland 0.5 turbidity standard. Subsequently, two inoculation levels of *Salmonella typhimurium* has been used to contaminate the meat samples. Inoculation level 10^2 CFU/g of meat has been considered as the low level inoculation and 10^8 CFU/g of meat has been considered as the high level *Salmonella typhimurium* inoculation.

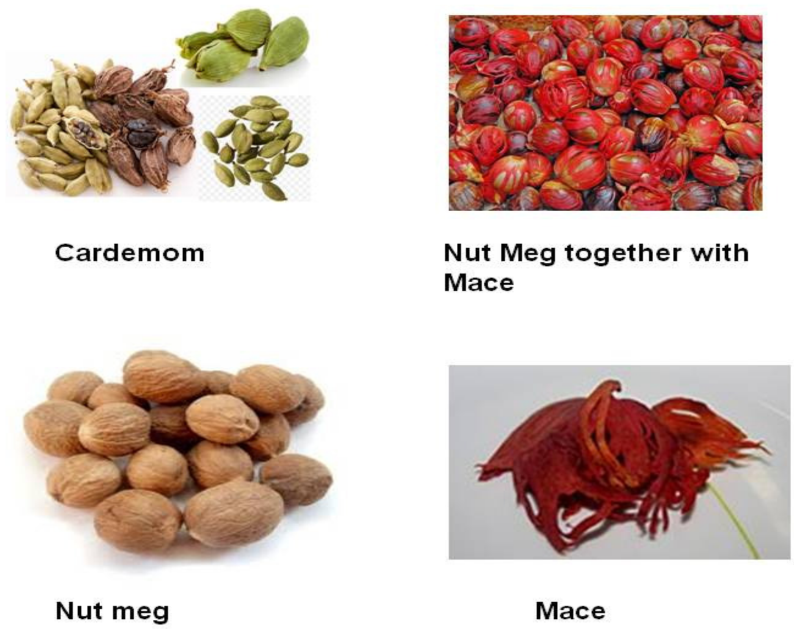

Fig. 1. Natural compounds used in the study

2.5 Contamination of Chicken Meat Samples

Frozen poultry meat portions were used for the study with a weight of 10± 0.2 g per sample and rinsed in sterile distilled water followed by 30 minutes of UV treatment to decontaminate the meat samples. Meat samples (n=9 for one treatment) were contaminated with two concentrations of *Salmonella typhimurium* (10^2 and 10^8 CFU/g) (mentioned above). Meat samples with three replicates were contaminated artificially by adding the respective number of bacteria in to the meat containing sterilized flask. Contaminated samples were shake in an automatic shaker for 30 minutes with continuous shaking (150 rpm/minute) and removed the culture medium containing bacteria and drained the meat samples well followed by the washing with sterilized distilled water. Meat samples without contamination with *Salmonella* were used as the uncontaminated control to see the sterility of meat samples.

2.6 Decontamination of Poultry Meat with Organic Chemicals and Natural Compounds

The experiment was consisted of two treatments as dipping of contaminated meat samples (10^2 and 10^8 CFU/g) in 1% solutions for 30 seconds which consisted of six replicates for each treatment. Each sample was immersed in the autoclaved flasks containing the 1% organic compounds such as citric, acetic, lactic acids and in 1% natural compounds such as Nutmeg, Mace and Cardamom for and 30 seconds and the flasks were agitated for respective time period (30 second) in a rotor having 200 rpm.

Fig. 2. Treatment of chicken meat with different natural and chemical compounds following the contamination with *S. typhimurium*

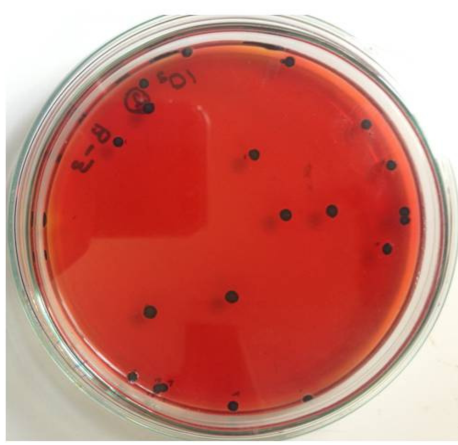

Fig. 3. Enumeration of *S typhimurium* colonies on Selective plates (XLD)

Contaminated meat samples without any chemical treatment were taken as the control in this study. Treated meat samples were grinded aseptically and were taken for the microbiological investigation. Experiment was performed twice to see the reproducibility.

2.7 Microbiological Investigation

Grinded meat samples were serially diluted in sterilized distilled water and 100 µl of the sample were plated on Xylose Lysine Deoxycholate agar (Himedia, India) plates using spread plate method in three replicates for each dilution. Following the inoculation plates were incubated at 35±2℃ for overnight and colonies were counted manually.

2.8 Statistical Analysis

Results were converted to logarithms and the analysis of data was carried out using the SAS statistical software (version 9.1, SAS Institute Inc. 2004). Data were compared with one way ANOVA, followed by a Tukey's post-hoc test. For all statistical analyses, a 5% significance level was used.

3. RESULTS AND DISCUSSION

The results of the study have shown that there is a statistically significant reduction of *Salmonella typhimurium* (ATCC 14028) colony count of meat samples at both of the inoculation levels (10^2 CFU/g of meat and 10^8 CFU/g of meat) when treated with Chemical and Natural compounds. Where the samples were treated with Chemical treatments Lactic, Acetic and Citric acids have shown 66% (25 CFU/g), 55% (33.33 CFU/g) and 51% (36.6 CFU/g) reduction of colony count at 10^2CFU/g inoculation and 88% (8.13×10^6CFU/g), 87% (9.3×10^6CFU/g) and 72% (2.03×10^7CFU/g) reduction at 10^8CFU/g inoculation level compared to the untreated control. And the degree of reduction has been elevated for the same treatment, with the elevation of the degree of inoculation. So at the 10^8 CFU/g inoculation, it has shown higher reduction values respectively for Lactic, Acetic and Citric acid Compared to the untreated control (Table 1).

Salmonella prefers pH in between 6.5 – 7.5 [15], so they have the tendency of depleted their biological function under extreme pH levels and possible eradication. All the acids used as treatments are having lower pH levels/ Acidic pH levels. At the 25℃ and 1 atm pressure, Lactic, Acetic and Citric acids are shown following pH values 3.51, 3.91 and 4.18 that are adverse to the growth of *Salmonella* species. This might be one of the possible reasons for the reduction of final colony count for all 3 treatments. According to the results obtained, it also showed that the increase of the acidity has increased the reduction of the final colony count. So this may be the reason for different reduction percentages for the different acids. It has shown a relationship between pH and reduction percentage, that the Higher the acidity/Lower the pH, will be more effective than treatment with a low acidity. As Lactic acid had the highest acidity/ lowest pH of the used treatments, it has shown the highest reduction percentage (66%) of the final colony count (Table 2).

Table 1. Effect of weak acids and natural compounds on *Salmonella typhimurium* at two different contamination levels (10^2 and 10^8 CFU/g of meat)

Meat contamination levels		**10^2 CFU/g of meat**		**10^8 CFU/g of meat**	
Treatments with organic acids and natural compounds		**Colony count (CFU/g of meat)**	**Reduction %**	**Colony Count (CFU/g of meat)**	**Reduction %**
Organic acids	Control	$75^{a} \pm 2.4$	0	$7.3 \times 10^{7\,a} \pm 5.09 \times 10^5$	0
	Lactic	$25^{b} \pm 5.0$	66.66	$8.13 \times 10^{6\,b} \pm 1.6 \times 10^5$	88.85
	Acetic	$33.33^{b} \pm 2.0$	55.55	$9.3 \times 10^{6\,b} \pm 5.4 \times 10^5$	87.26
	Citric	$36.66^{b} \pm 3.7$	51.11	$2.03 \times 10^{7\,c} \pm 1.36 \times 10^6$	72.12
Natural compounds	Control	$70^{p} \pm 4.4$	0	$7.2 \times 10^{7\,a} \pm 9.57 \times 10^5$	0
	Nutmeg	$10^{r} \pm 4.0$	85.71	$7.3 \times 10^{6\,b} \pm 1.06 \times 10^5$	89.77
	Mace	$11.6^{r} \pm 2.0$	83.33	$7.5 \times 10^{6\,b} \pm 1.49 \times 10^5$	89.58
	Cardamom	$23.3^{q} \pm 3.7$	66.66	$1.96 \times 10^{7\,c} \pm 5.9 \times 10^5$	72.78

Bacterial counts are expressed as Colony Forming Units/g ± Standard error). Values in the same column with a different superscript letter are significantly different from each other (P < 0.05) for each treatment (Acid treatment and natural compounds)

Table 2. Reduction percentages and pH levels (10^2 and 10^8 CFU/g of meat)

Treatment	pH	Percentage reduction 10^2 CFU	Percentage reduction 10^8 CFU
Lactic acid	3.51	66	88
Acetic acid	3.91	55	87
Citric acid	4.18	51	72

The influence of three organic acid, (acetic, lactic and citric acid in different concentrations) were evaluated over two major foodborne pathogens, *Listeria monocytogenes* and *Salmonella enteritidis* by Jankuloski and his group revealed the effect of these acids to reduce the bacterial growth [16]. In another experiment, done by Menconi and the colleagues and found that blend of organic acids such as acetic, citric and propionic acids have shown a significant antibacterial activity against three foodborne pathogens namely, *S. typhimurium*, *Escherichia coli* O157:H7, or *Listeria monocytogenes* commonly implicated in meat processing [17]. Further supporting the current study there were many findings in the literature who found that antibacterial effect against *S. thypimurium* [18-21].

3.1 Effect of Natural Compounds on *Salmonella typhimurium*

When it comes to the natural treatments, it is comparatively health beneficial, as natural compound is not showing any harmful effects on humans. But it is not economically friendly as they all are expensive than the chemical treatments. Samples that were treated with Natural compounds, such as Cardamom, Nutmeg and Mace were also shown a significance level of reduction of the Salmonella colony count. At the level of 10^2 CFU/g inoculation level, all the treatments (Cardamom, Nutmeg and Mace) have shown 85% (10 CFU/g), 83% (11.6 CFU/g) and 66% (23.3 CFU/g) reduction and 89% ($7.3x10^6$ CFU/g), 89% ($7.5x10^6$ CFU/g) and 72% ($1.96x10^7$ CFU/g) reduction at 10^8CFU/g inoculation level (Table 1).

Cardamom consists a complex blend of natural oils and therefore it has many organic compounds that give certain characteristics to Cardamom, such as aromatic, antiseptic, stimulant, carminative, stomachic, expectorant, anti-spasmodic and diuretic abilities [22]. Among many other organic molecules, majority of Cardamom's natural oil mainly consist with 36.3% 1,8-cineole, 31.3% α-terpinyl acetate, 11.6% limonene, 3% linalool, 2.8% sabinene, 2.7% trans-nerolidol, 2.6% α-terpineol, 2.5% linalyl acetate, 1.6% myrcene, 1.5% α-pinene [14]. Major constituents of Cardamom have shown antimicrobial effects on several occasions [23]. This might be a possible reason behind the significant reduction of *Salmonella typhimurium* colony count in this study.

On the other hand, Nutmeg and Mace are derived from the same fruit. Nutmeg is the kernel inside the seed and mace is the soft outer layer of the "Nutmeg fruit". So they are sharing common characteristics when it comes to their chemical compositions. Yet, there are slight differences too. Nutmeg seed oil mainly consists of 21.38% sabinene, 13.92% 4-terpineol and 13.57% myristicin [24]. Nutmeg contains 35.4% total lipids, 74.9% of which is myristic acid. Also, Mace contains 30.4% total lipids and out of that amoount 40.3% is oleic acid [25].

Sabinene has been identified as an antimicrobial compound. Sabinene is a natural bicyclic monoterpene with the molecular formula $C_{10}H_{16}$. It is isolated from the essential oils of a variety of plants including holm oak (*Quercus ilex*), Norway spruce (*Picea abies*), Nutmeg (*Myristica fragrans)* [26]. It has successfully controlled the growth of *Escherichia coli* ATCC 25922 and *Enterobacter aerogenes* [27]. Also sabinene has pronounced anti-inflammatory and antibacterial properties [28-30]. As both Nutmeg and Mace are in rich with sabinene, it might be one of the many reasons to reduce the final *Salmonella* count. In agreement with the current study Uhart and the colleagues found that natural products such as ginger, garlic and turmeric inhibit or inactivate the growth of *Salmonella Typhimurium* DT 104 in beef [31]. Further in line with the current study, the effects of coriander, garlic, rosemary, and orange peel oils on the survival of *Salmonella* Enteritidis was studied by Tosun and the group and they confirm that these natural products have antibacterial effect against the organism [32]. A stud done with oregano and cinnamon essential oils and powdered olive and apple extracts against *Salmonella enterica* serovar Typhimurium DT104 in ground pork showed the possibility of controlling the Salmonella by these natural compounds and it further supports the findings of this study [33].

Many other research found the effect of natural compounds against salmonella confirming the findings of this study [34-37].

4. CONCLUSION

It can be concluded that the natural compounds (nutmeg, mace and cardamom) and organic acids (acetic, citric and lactic) have an antibacterial effect on *S. typhimurium*, and, therefore, it can be used for the decontamination process of poultry meat. The natural compounds have shown greater efficiency in destroying the *S. typhimurium* compared to the chemical compounds.

DISCLAIMER

I hereby declare that part of this work was previously published as an extended abstract in the following conference.

Name of the conference: International Symposium on Agriculture and Environment 2014 (ISAE 2014).
Venue of the conference: University of Ruhuna, Sri Lanka
Date of the Conference: 27 November 2014
https://www.cabdirect.org/cabdirect/abstract/20153384251

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ceylon Grain Elevators PLC. Annual Report; 2011. Available:http://www.prima.com.lk/data/pdf/ceylon_grain_elevators_annual_report_2011.pdf
2. Müller O, Krawinkel M. Malnutrition and health in developing countries. CMAJ : Canadian Medical Association Journal. 2005;173(3):279-286. DOI: 10.1503/cmaj.050342
3. Lynch MF, Tauxe RV, Hedberg CW. The growing burden of foodborne outbreaks due to contaminated fresh produce: Risks and opportunities. Epidemiol. Infect. 2009;137:307–315.
4. Hanning IB, Nutt JD, Ricke SC. Salmonellosis outbreaks in the United States due to fresh produce: Sources and potential intervention measures. Foodborne Pathogens and Disease. 2009; 6(6):635-648.
5. Sivapalasingam S, Friedman CR, Cohen L, Tauxe RV. Fresh produce: A growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. Journal of Food Protection. 2004; 67(10):2342-53.
6. Fearnley E, Raupach J, Lagala F, Cameron S. Salmonella in chicken meat, eggs and humans; Adelaide, South Australia, 2008. Int J Food Microbiol. 2011; 146(3):219-27.
7. Hwang H, Dwyer J, Russell RM. Diet, Helicobacter pylori infection, food preservation and gastric cancer risk: Are there new roles for preventative factors?. Nutrition Reviews. 1994;1:52(3):75-83.
8. Jackson BR, Griffin PM, Cole D, Walsh KA, Chai SJ. Outbreak-associated *Salmonella enterica* serotypes and food commodities, United States, 1998–2008. Emerging Infectious Diseases. 2013; 19(8):1239.
9. Sandigawad AM. Analysis of phytochemicals and antibacterial potential of *Lonicera japonica* Thunb. Int. J. Pharm. Bio. Sci. 2015;6:571–583.
10. McEwen SA, Fedorka-Cray PJ. Antimicrobial use and resistance in animals. Clinical Infectious Diseases. 2002;1;34(Supplement_3):S93-106.
11. Jayasena DD, Jo C. Essential oils as potential antimicrobial agents in meat and meat products: A review. Trends in Food Science & Technology. 2013;1;34(2):96-108.
12. Rattanachaikunsopon P, Phumkhachorn P. Lactic acid bacteria: Their antimicrobial compounds and their uses in food production. Annals of biological research. 2010;1(4):218-28.
13. Rhee MS, Lee SY, Dougherty RH, Kang DH. Antimicrobial effects of mustard flour and acetic acid against Escherichia coli O157: H7, Listeria monocytogenes, and Salmonella enterica serovar *typhimurium*. Applied and environmental microbiology. 2003;1:69(5):2959-63.
14. Killinger KM, Kannan A, Bary AI, Cogger CG. Validation of a 2 percent lactic acid antimicrobial rinse for mobile poultry slaughter operations. Journal of food protection. 2010;73(11):2079-83.
15. Huiying L. The antimicrobial effects of weak acids on *Escherichia coli* O157: H7,

and the application of fumaric acid to develop processing treatments for 5-log reduction of *Escherichia coli* O157: H7 in refrigerated pickle products. Master thesis; 2011.
Available:http://www.lib.ncsu.edu/resolver/1840.16/6841
16. Jankuloski D, Sekulovski P, Angelovski L, Prodanov M, Ratkova M, Kostova S. Effect of acetic, citric and lactic acid on salmonella enteritidis and listeria monocytogenes. Journal of Hygienic Engineering and Design. 2014;93-95.
17. Menconi A, Shivaramaiah S, Huff GR, Prado O, Morales JE, Pumford NR, Morgan M, Wolfenden A, Bielke LR, Hargis BM, Tellez G. Effect of different concentrations of acetic, citric, and propionic acid dipping solutions on bacterial contamination of raw chicken skin, Poultry Science. 2013;92:8(1):2216–2220.
Available:https://doi.org/10.3382/ps.2013-03172
18. Harris K, Miller MF, Loneragan GH, Brashears MM. Validation of the use of organic acids and acidified sodium chlorite to reduce Escherichia coli O157 and *Salmonella Typhimurium* in beef trim and ground beef in a simulated processing environment. J. Food Prot. 2006;69:1802–1807.
19. Gonzalez-Fandos E, Herrera B. Efficacy of acetic acid against *Listeria monocytogenes* attached to poultry skin during refrigerated storage. Foods. 2014; 3(3):527-540.
DOI: 10.3390/foods3030527
20. Kang S, Jang A, Lee SO, Min JS, Kim IS, Lee M. Effect of organic acids on microbial populations and *Salmonella typhimurium* in pork loins. Asian-Australas J Anim Sci. 2003;16:96–99.
21. Jang Y, Lee K, Yun S, Lee M, Song J, Chang B, Choe NH. Efficacy evaluation of commercial disinfectants by using Salmonella enterica serovar Typhimurium as a test organism. J Vet Sci. 2017; 18(2):209-216.
Available:https://doi.org/10.4142/jvs.2017.18.2.209
22. Alakomi HL, Skyttä E, Saarela M, Mattila-Sandholm T, Latva-Kala K, Helander IM. Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. Applied and Environmental Microbiology. 2000;1:66(5):2001-5.
23. Kokab S, Ahmad S. Developing herbal pharmaceutics in Pakistan–II: Distinctiveness of selected medicinal herbs and uses. National Resourse Division, Pakistan Agriculture Research Council, Islamabad, Pakistan. 2011;3:7-8.
24. Ağaoğlu S, Dostbil N, Alemdar S. Antimicrobial effect of seed extract of cardamom (Elettarıa cardamomum Maton). YÜ Vet Fak Derg. 2006;16(2):99-101.
25. Hendry ER, Worthington T, Conway BR, Lambert PA. Antimicrobial efficacy of eucalyptus oil and 1, 8-cineole alone and in combination with chlorhexidine digluconate against microorganisms grown in planktonic and biofilm cultures. Journal of Antimicrobial Chemotherapy. 2009; 16:64(6):1219-25.
26. Subarnas A, Apriyantono A, Mustarichie R. Identification of compounds in the essential oil of nutmeg seeds (Myristica fragrans Houtt.) that inhibit locomotor activity in mice. International Journal of Molecular Sciences. 2010;23:11(11):4771-81.
27. Parthasarathy VA, Chempakam B, Zachariah TJ, editors. Chemistry of Spices. Cabi; 2008.
28. Fischbach RJ, Zimmer I, Steinbrecher R, Pfichner A, Schnitzler JP. Monoterpene synthase activities in leaves of Picea abies (L.) Karst. and Quercus ilex L. Phytochemistry. 2000;1:54(3):257-65.
29. Sağdıç O, Özkan G, Özcan M, Özçelik S. A study on inhibitory effects of sığla tree (*Liquidambar orientalis* Mill. var. orientalis) storax against several bacteria. Phytotherapy Research. 2005;1:19(6):549-51.
30. Ocana-Fuentes A, Arranz-Gutierrez E, Senorans FJ, Reglero G. Supercritical fluid extraction of oregano (Origanum vulgare) essentials oils: Anti-inflammatory properties based on cytokine response on THP-1 macrophages. Food and Chemical Toxicology. 2010;1:48(6):1568-75.
31. Uhart M, Maks N, and Ravishankar S.Effect of spices on growth and survival of *Salmonella Typhimurium* DT 104 in ground beef stored at 4 and 8 8C. Journal of Food Safety. 2006;26:115–125.
Available:https://doi.org/10.1111/j.1745-4565.2006.00036.x

32. Tosun ŞY, Uçok Alakavuk D, Ulusoy Ş, Erkan N. Effects of essential oils on the survival of *Salmonella enteritidis* and *Listeria monocytogenes* on fresh *Atlantic salmons* (Salmo salar) during storage at 2 ℃ 1 8℃. J Food Saf. 2018; 38:e12408. Available:https://doi.org/10.1111/jfs.12408
33. Chen CH, Ravishankar S, Marchello J, Friedman M. Antimicrobial activity of plant compounds against *Salmonella typhimurium* DT104 in Ground Pork and the Influence of Heat and Storage on the Antimicrobial Activity. Journal of Food Protection. 2013; 76:7:1264-1269.
34. Indu MN., Hatha A, Abirosh C, Harsha U, Vivekanandan G. Antimicrobial activity of some of the south-Indian spices against serotypes of *Escherichia coli*, *Salmonella*, *Listeria monocytogenes* and *Aeromonas hydrophila*. Braz. J. Microbiol. 2006; 37:153–158. DOI: 10.1590/S1517-83822006000200011
35. Thongson C, Davidson PM, Mahakarnchanakul W, Vibulsresth P. Antimicrobial effect of Thai spices against *Listeria monocytogenes* and *Salmonella typhimurium* DT104. J. Food Prot. 2005; 68:2054–2058. DOI: 10.4315/0362-028X-68.10.2054
36. Bisht DS, Menon K, Singhal MK. Comparative antimicrobial activity of essential oils of *Cuminum cyminum* L. and *Foeniculum vulgare* Mill seeds against *Salmonella typhimurium* and *Escherichia coli*. J. Essent. Oil Bear. Plants. 2014; 17:617–622. DOI: 10.1080/0972060X.2014.956675
37. Naveed R, Hussain I, Tawab A, Tariq M, Rahman M, Hameed S, Mahmood MS, Siddique A, Iqbal M. Antimicrobial activity of the bioactive components of essential oils from Pakistani spices against *Salmonella* and other multi-drug resistant bacteria. BMC Complement. Altern. Med. 2013;13:265. DOI: 10.1186/1472-6882-13-265

Peer-review history:
The peer review history for this paper can be accessed here:
http://prh.sdiarticle3.com/review-history/25165