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Research Article

Study on the efficacy of green tea extract on *Staphylococcus aureus* and *Klebsiella pneumonia* of raw chicken meat

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Abstract

We investigated the efficacy of green tea extract on *Staphylococcus aureus* and *Klebsiella pneumonia* of raw chicken meat. The experiment was conducted using complete randomized design (CRD) having six (6) treatments with three (3) replications. The germicidal activity was determined using the drop plate method. The results indicated that the addition of green tea extract (GTE) decreased microbial population in raw chicken meat. The lowest colony forming unit/ml (CFU ml^{-1}) was found in T_5 in 10^{-3} dilution factor (DF). There was a highly significant difference ($p<0.001$) in CFU $ml^{-1}$of *Staphylococcus aureus and Klebsiella pneumonia* among different treatments i.e., GTE has significant effect ($p<0.001$) on *Klebsiella pneumoniae and Staphylococcus aureus*. The maximum number of *Staphylococcus aureus* in raw chicken meat was 14×10^5 CFU ml^{-1}in T_0 using TVC, while minimum was 4×10^4 CFU ml^{-1}in T_5. Similar trend was also found in *Klebsiella pneumoniae*. GTE also showed highly significant ($p<0.001$) germicidal efficacy on both of *Staphylococcus aureus* and *Klebsiell.* The GTE also showed significant ($p<0.01$) germicidal efficacy on *Staphylococcus epidermidis, Salmonella spp., Shigella spp.* and *E. coli*. Considering the results obtained, it might be concluded that addition of 5 ml GTE reduce microbial population of raw chicken.

Introduction

Meat is a highly perishable food due to its high nutritive value for microbes. Poultry meat and products have frequently been found to be contaminated with microorganisms during the butchering and manufacturing process **(**Huiyun et al., 2015). Chicken meat is favored by consumers around the world because of its desirable nutritional qualities, such as low-fat content, high concentration of polyunsaturated fatty acids (PUFA) (Patsias et al., 2008; Bithi et al., 2020; Disha et al., 2020) and cheaper than other meat (Guerrero, 2010). Addition of plant extract or anti-oxidant during the processing of meat product increases the quality and self life of the products (Akter et al., 2022; Ali et al., 2022; Azad et al., 2021; Hossain et al., 2021). Nowadays, there is an increasing interest in the biochemical functions of natural antioxidant extracts from vegetables, fruits, and medicinal plants, which can become candidates to prevent oxidative damage, promoting health. Consumers' attention has focused on health problems associated with microbial contamination and are looking for microbial safe meat and products, preserved with natural substances rather than synthetics (Islam et al., 2021; Khatun et al., 2022; Saba et al., 2018; Sadakuzzaman et al., 2021; Siddiqua et al., 2018). Microbial growth in meat results in slime formation, structural components degradation, decrease in water holding capacity, off odors and texture and appearance changes (Dave and Ghaly, 2011). To extend the shelf life of chicken meat and products, wide range of natural bio preservatives e.g. essential oils and various extracts of plants are used, and are therefore, outstanding substitution of chemical preservatives (Burt, 2004; Pajohi et al., 2011; Boby et al., 2021). The catechins and caffeine, the main components of GTE are the compounds attributed to the antimicrobial activity. The caffeine of the tea is responsible for inhibition of normal cell division thus inhibiting spore germination and growth of microbes (Aneja and Gianfagna, 2001). GTE demonstrated inhibitory properties against major pathogens including *L. monocytogenes, E. coli, Salmonella typhimurium, Campylobacter jejuni*, and others including *Staphylococcus aureus, Staphylococcus epidermidis, Salmonella Enteriditis, Shigella flexneri, Shigella dysenteriae*, and *V. cholera* (Toda et al., 1991; An et al., 2004; Gadang et al., 2008). Quality deterioration of meat has thrown a serious challenge in developing countries like Bangladesh. In spite of the increased consumer demand on food safety standards for chicken in Bangladesh, there are still poor hygienic and sanitary practices along the meat production chain which leading to growth of unacceptable level of microbial population. Extensive review of literature reveals that very scanty research work has been done in Bangladesh on the microbial population of raw chicken using GTE. The present study aims to investigate the efficacy of GTE on *Staphylococcus aureus* and *Klebsiella pneumonia* of raw chicken meat.

Materials and Methods

Collection of raw materials

Chicken weighing 100 gm after 1-2 h post-mortem, was purchased from Gollamari Bazar, Khulna of Khulna City Corporation. Green tea was purchased from super shop, Safe 'n' Save in Khulna City.

Bacteriological media

Media used for bacteriological analysis, detection, count and identification of *Staphylococcus aureus* and *Klebsiella pneumonia* in chicken meat were Mannitol Salt Agar (MSA), Xylose Lysine Deoxycholate (XLD) Agar and Eosin Methylene Blue (EMB) Agar.

Experimental design

The experiment was conducted using a completely randomized design (CRD) with six (6) treatments (T_0 =0 ml GTE; T_1 =1 ml GTE; T_2 =2 ml GTE; T_3 =3 ml GTE; T_4 =4 ml GTE; T_5=5ml GTE) having three (3) replications. Six different meat mixes (1gm meat for each treatment) were prepared; control was prepared with 1gm chicken meat and 10 ml normal saline (0.9% NaCl) solution.

Sample preparation

The chicken meat was washed in normal saline (0.9% NaCl) solution. For control 1gm meat sample was added with 1ml (1000 µl) saline and grinded it properly. After grinding 9 ml saline solution was added. 1gm of meat sample was soaked in 1ml green tea extract (30 minute). Then 10 ml saline was added and grinded it properly. Following grinding, chicken meat was assigned for six treatments. GTE was prepared and collected as described by Sarah et al. (2010).

Methods used for laboratory analysis

Monitoring parameter (TVC)

1gmmeat sample for each treatment was weighed and put in T_0, T_1, T_2, T_3, T_4, T_5. T_0 was left as the fresh sample while T_1, T_2, T_3, T_4 and T_5 were subjected to surface treatment using 1ml, 2ml, 3ml, 4 ml and 5ml of GTE, respectively. All samples were stored at room temperature (25 $^{\circ}$C) and were analyzed immediately 30 minutes after treatment application.

Sample plating (by using drop plate method)

A series of dilutions of the sample were prepared. Then two sterilized Petri dishes were taken and agar medium was poured into them and allowed to dry. Each agar plate was divided into four quadrants by marking over the lid with a glass marking marker. Each quadrant was reserved for one dilution in the series. Then the plates were arranged, sample was drawn by the pipette from highest dilution first (10^{-7}), the segment of the plate marked 10^{-7} was brought nearer, the lid was lifted on one side and a drop of suspension was delivered from the pipette aseptically on the labeled segment. The electronic pipette was programmed to pick up 10 µl. The first dilution tube was vortexed for approximately 6s and 10µl was picked up using the electronic pipette. Eighty microliters were dispensed in 8 evenly spaced 10µl drops onto the designated quadrant of the Petri plate. The tip and the remaining sample were discarded. Same procedure was followed for duplicate Petri plate. After the drops on the agar dried, the Petri plates were inverted and incubated at 37 $^{\circ}$C for 18–24 hrs.

Observation

While the incubation period was over the microorganisms were grown in the Petri plates and counted in CFU ml^{-1}via visual method.

Enumeration of TVC, *Klebsiella pneumonia* count (KPC) and *Staphylococcus aureus* count (SAC)

TVC, SAC, and KPC in the samples were counted following the methods of viable count of bacteria by Drop Technique with suitable modifications whenever necessary. Viable bacteria were counted by diluting samples, plating the dilutions on solid medium and counting the colonies that arise. Results were usually expressed as colony-forming units (CFU). The number of CFU per gram of original sample was calculated by using the formula:

Number of CFU ml^{-1} (or g^{-1}) = $N \times 10^n \times 10$

Where, N = no. of colonies on the plate at the selected dilution.

n = no. of dilution.

Total Viable Count (TVC)

For evaluating Total Viable Count, the drop plate technique was followed using 10^{-1} to10^{-7} dilutions. The plates containing between 5-50 colonies in consecutive dilutions were selected to calculate the results.

Klebsiella pneumonia count (KPC)

Klebsiella pneumonia was isolated and enumerated using XLD agar (Hi Media, Mumbai, India). Briefly, 10 µl of 10^{-1} to 10^{-7} dilution were drop plated on dried plates of XLD agar and incubated at 37˚C for 18-24 hrs. The presumptive colonies were determined by counting number of large yellow gummy colonies. The colonies were confirmed by streaking 2-3 colonies on to EMB (Levine) agar and colonies of *Klebsiella* species produces large, mucoid, pink to purple colonies with no metallic green sheen on EMB agar.

Staphylococcus aureus count (SAC)

MSA was used for isolation and enumeration of *Staphylococcus aureus*. Briefly, 10 µl of 10^{-1} to 10^{-7} dilution were drop plated on dried plates of MSA (Hi-Media Mumbai, India) and incubated at 37˚C for 18-24 hrs. The presumptive colonies were determined by yellow colonies with yellow zones and by streaking 2-3 colonies on MSA for better view.

Identification of *Staphylococcus aureus*

From MSA plate, 3-4 presumed colonies with characteristics suggestive for *Staphylococcus aureus* were selected and streaked on MSA. Isolates having yellow color colonies on MSA after incubation for 24 hr. at 37 °C were presumed to be *Staphylococcus aureus.*

Identification of *Klebsiella pneumoniae*

From XLD agar plate, large yellow gummy color colonies were presumed to be *Klebsiella pneumoniae*. Three to four assumed colonies were then streaked on EMB agar with incubation for 24 hr. at 37 °C. A large, mucoid, pink to purple colonies with no metallic green sheen on EMB agar indicates the presence of *Klebsiella pneumoniae*.

Statistical analysis

Data were analyzed using the GLM procedure of SAS version 9.1, (SAS Institute, Inc; 1996). DMRT was used to compare the treatment means with significance considered at $p<0.05$.

Results and Discussion

Bacteria count of raw chicken meat

The CFU ml^{-1} of *Staphylococcus aureus* were similar in the different treatments except 30.00 in T_5 in the dilution factor (DF) of 10^{-1}; but in10^{-2} DF, the CFU ml^{-1} was gradually decreased in Figure 1. The lowest CFU $ml^{-1}$was found in T_5 with 10^{-3} DF. The CFU ml^{-1} of *Klebsiella pneumoniae* were also similar in the different treatments except 30.00 in T_4 and 20 in T_5 in DF of 10^{-1}, respectively; but in10^{-2} and10^{-3} DF, the CFU ml^{-1} was gradually decreased. The lowest CFU ml^{-1} was found in T_5 in 10^{-3} DF.CFU ml^{-1} of *Staphylococcus aureus* and *Klebsiella pneumonia* in raw chicken meat was highly significantly ($p<0.001$) differed among the treatments and was agreed with findings of Radji et al. (2006) who reported that green tea have antimicrobial activities against various pathogenic bacteria. Similar results were also obtained by Gadang et al. (2008) who reported that the liquid extracts of green tea seed have shown inhibitory effects on some gram positive (*Staphylococcus aureus*) as well as gram negative (*Klebsiella pneumoniae*) bacteria. The present findings were also consistent with the observation of Sivarooban et al. (2008) who reported that tea catechins extracted from green tea have shown inhibitory effect on gram positive (*Staphylococcus aureus*) and gram negative (*Klebsiella pneumoniae*) bacteria (Cao et al., 2005; Hara-Kudo et al., 2005).

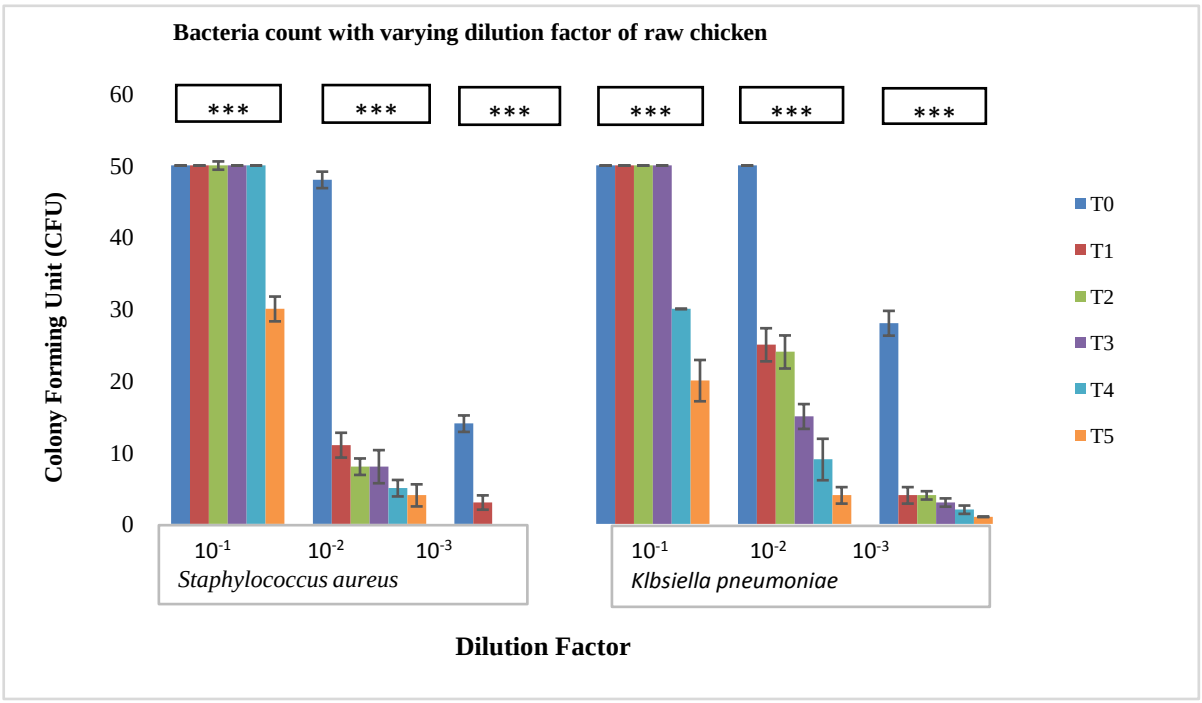

Fig 1. Bacteria count with varying dilution factor of raw chicken.

Average colony forming unit/ml of bacteria

In this study, the inhibitory effects of different concentrations of GTE on the growth of the tested bacteria of raw chicken are shown in Figure 2. Initial mean value of *Staphylococcus aureus* and *Klebsiella pneumoniae* was highest in T_0 while gradually decreasing the number of both organisms in T_5. It clearly reveals that average CFU ml^{-1} decreased highly significantly ($p<0.001$) with addition of more GTE into raw chicken meat. We found similarity with the findings of Jasim et al. (2011) that GTE were active against *E. coli, Streptococcus pneumoniae, Klebsiella pneumoniae, Staphylococcus aureus*, Proteus species and *Pseudomonas aeruginosa*. Similar results were also found by Sivarooban et al. (2008) who reported similar observation that tea catechins extracted from green tea have shown inhibitory effects on gram positive (*Staphylococcus aureus*) and gram negative (*Klebsiella pneumoniae*) bacteria (Toda et al., 1991; An et al., 2004; Gadang et al., 2008)

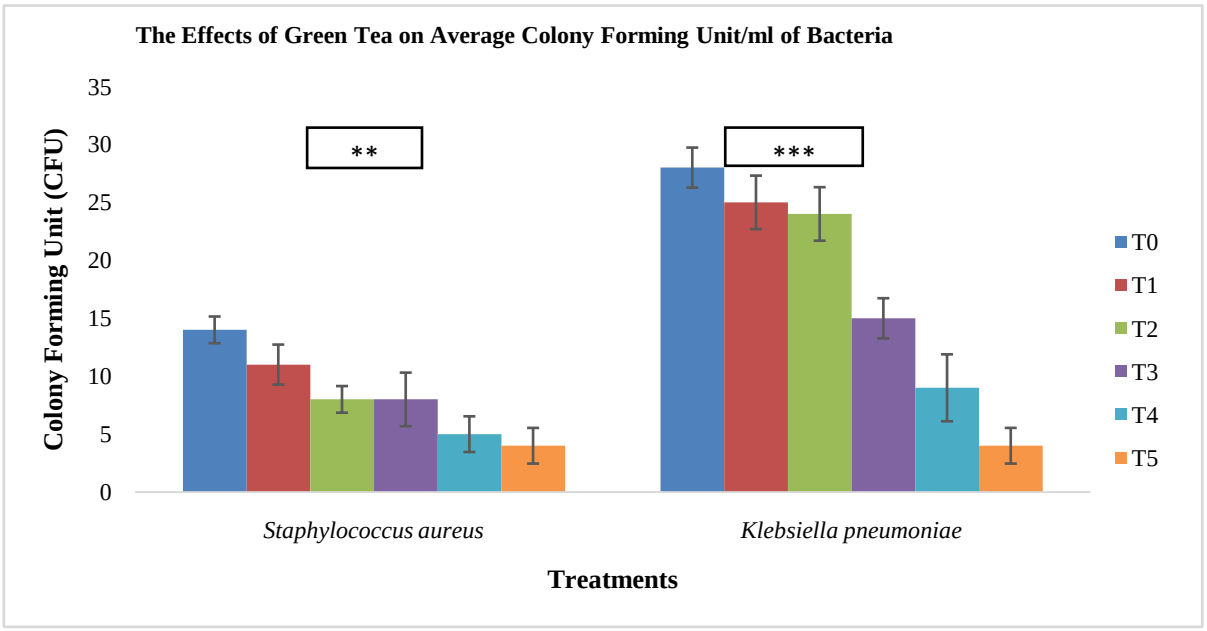

Fig 2. The effects of green tea on average colony forming unit/ml of bacteria.

TVC of bacteria in raw chicken

The TVC of microbes in raw chicken meat was shown in Figure 3. Highest TVC of *Staphylococcus aureus* in raw chicken meat was found in T_0, while minimum in T_5. Similar trend was observed for *Klebsiella pneumonia* of raw chicken meat. Highest TVC was 28×10^5CFU ml^{-1} in T_0 and gradually reduced to minimum 4×10^4 CFU ml^{-1} in T_5. The TVC in raw chicken meat was highly significantly (p<.001) varied among different treatments.

GTE showed highly significant difference in their antimicrobial activity against both *Staphylococcus aureus* and *Klebsiella pneumoniae* and showing similar growth inhibition against both. We found similarity with the findings of Jasim et al. (2011) that GTE were active against *E. coli*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Proteus species* and *Pseudomonas aeruginosa* (Toda et al., 1991; An et al., 2004; Gadang et al., 2008).

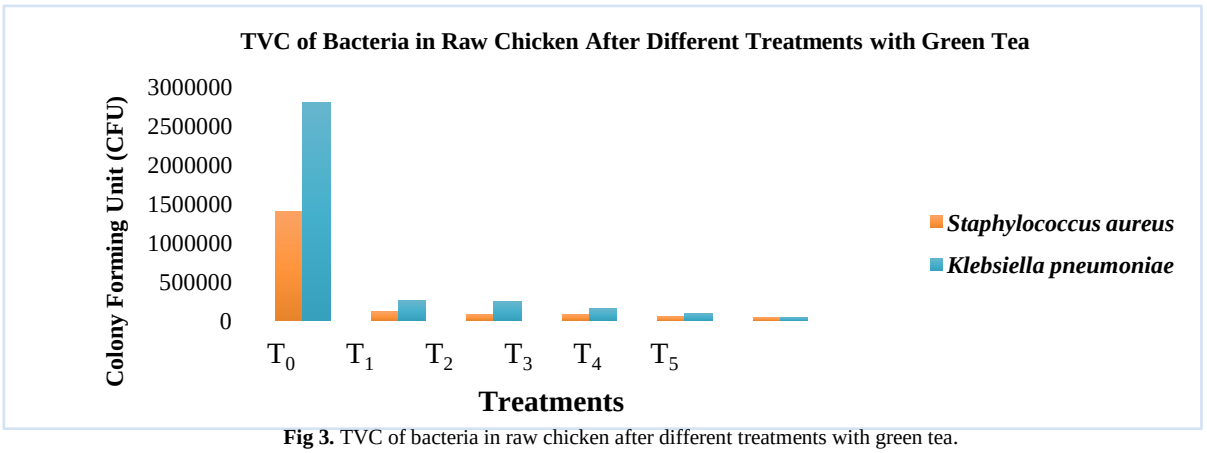

Fig 3. TVC of bacteria in raw chicken after different treatments with green tea.

Antimicrobial activity of GTE against *Staphylococcus epidermidis, Salmonella spp., Shigella spp., and E. coli*

The bacteria counted in this experiment included *Staphylococcus epidermidis*, *Salmonella spp.*, *Shigella spp.*, and *Escherichia coli* shown in Table 1. The average CFU ml^{-1} of *Staphylococcus epidermidis* was 20 and 2 in T_0 and T_5. Similar trend was also observed in the *Shigella spp* where 5 and 2 CFU ml^{-1} was found in in T_0 and T_5.Four (4) and 2 CFU ml^{-1} of *Salmonella spp.* was also found in T_0 and T_1; and 2 CFU ml^{-1} of *Escherichia coli* was found in T_0. There is no growth of colony forming unit in T_1, T_2, T_3, T_4, and T_5 for *Escherichia coli*. It reveals that average CFU ml^{-1} was decreased with addition of increased GTE into raw chicken meat. The present result was consistent with the findings of Toda et al. (1991), An et al. (2004) and Gadang et al. (2008) who reported that GTE had profound inhibitory properties against major pathogens including *L. monocytogenes*, *E. coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Campylobacter jejuni*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella enteriditis*, *Shigella flexneri*, *Shigella dysenteriae*, and *V. cholera*. Experiment conducted by Radji et al. (2006) showed that GTE have antimicrobial activities against various pathogenic bacteria which satisfy the present findings. Similar results were also obtained in the findings of the several researchers who reported that GTE is one of the undoubted potent natural preservatives applied to many foods as antioxidant and antimicrobial agents (Wang and Zhao, 1997; Tang et al., 2001; An et al., 2004; Mitsumoto et al., 2005; Zhu et al., 2005; Su et al., 2008; Kristanti and Punbusayakul, 2009).

Table 1. Antimicrobial activity of green tea against CFU/ml of *Staphylococcus epidermidis, Salmonella spp., Shigella spp., and E. coli*

CFU/ml of Bacteria	Treatments						Sig. Level
	T_0 (Mean ±SE)	T_1 (Mean ±SE)	T_2 (Mean ±SE)	T_3 (Mean ±SE)	T_4 (Mean ±SE)	T_5 (Mean ±SE)	
S. epidermidis	20^{a}±2.89	12^{ab}±1.54	11^{b}±0.58	5^{bc}±3.46	2^{c}±4.62	2^{c}	**
Salmonella spp.	4^{a}±1.54	2^{b}±0.58	0	0	0	0	***
Shigella spp.	5^{a}±0.33	3^{b}±0.58	3^{b}±0.58	3^{b}±0.58	2^{b}	2^{b}	**
E. coli	2^{a}±1.15	0	0	0	0	0	*

T_0 (0 ml of GT), T_1 (1 ml of GT), T_2 (2 ml of GT), T_3 (3 ml of GT), T_4 (4 ml of GT), T_5 (5 ml of GT), NS= Non-significant; *= p<.05; **= p<0.01; ***= p<0.0001. Mean with different superscripts within same row differ significantly (p<.0001).

Conclusion

Addition of GTE to raw chicken meat restricts the growth of microbese.g. *Staphylococcus aureus* and *Klebsiella pneumoniae*. Gradual increased amount of GTE significantly decreased the amount ofmicrobial load. Therefore, it might be concluded that addition of 5 ml GTE (T_5) can be used in raw chicken meat to preserve it through reduction of microbial load.

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Conflicts of Interest

The authors declare that there are no potential conflicts of interests.

References

Akter R, Hossain MA, Khan M, Rahman MM, Azad MAK, Hashem MA. 2022. Formulation of value-added chicken meatballs by addition of Centella leaf (*Centella asiatica*) extracts. Meat Research, 2: 2, Article No. 18. https://doi.org/10.55002/mr.2.2.18

Ali MS, Rahman MM, Habib M, Kabir MH, Hashem MA, Azad MAK, Rahman MM. 2022. Quality of spent hen sausages incorporated with bee honey. Meat Research, 2: 1, Article 9. https://doi.org/10.55002/mr.2.1.9

An BJ, Kwak JH, Son JH, Park JM, Lee JY, Jo C, Byun MW. 2004. Biological and anti-microbial activity of irradiated green tea polyphenols. Food Chemistry 88(4), 549–555.

Aneja M, Giafagna T. 2001. Induction and accumulation of caffeine in young, actively growing leaves of cocoa (Theobroma cacao L) by wounding or infection with Crinipellisperniciosa. Physiol. Mol. Plant Pathol., 59: 13-16.

Azad MAK, Kikusato M, Zulkifli I, Rahman MM, Ali MS, Hashem MA, Toyomizu M. 2021. Comparative study of certain antioxidants - electrolyzed reduced water, tocotrienol and vitamin E on heat-induced oxidative damage and performance in broilers. Meat Research, 1: 1, Article 7. DOI: https://doi.org/10.55002/mr.1.1.7

Bithi MAA, Hossain MA, Rahman SME, Rahman MM, Hashem MA. 2020. Sensory, nutritive, antioxidant and antimicrobial activity of telakucha (*Coccinia cordifolia*) leaves in broiler meatballs. Journal of meat Science and Technology, 8(2): 23-31.

Boby F, Hossain MA, Hossain MM, Rahman MM, Azad MAK, Hashem MA. 2021. Effect of long coriander leaf (*Eryngium fotidum)* extract as a natural antioxidant of chicken meatballs during at freezing temperature. SAARC Journal of Agriculture, 19(2): 271-283.

Burt S. 2004. Essential oils: Their antibacterial properties andpotential applications in foods – A review. Int. J.FoodMicrobiol.**,**94**,** 223–253.

Cao BH, Karasawa Y, GuoYM. 2005. Effects of green tea polyphenols and fructo-oligosaccharides in semi-purified diets on broilers performance and caecal microflora and their metabolites. Asian-Aust. J. Anim. Sci., 18:85-89.

Dave D and Ghaly AE. 2011. Meat Spoilage Mechanisms and Preservation Techniques: A Critical Review. American Journal of Agricultural and Biological Sciences, 6 (4): 486-510.

Disha MNA, Hossain MA, Kamal MT, Rahman MM, Hashem MA. 2020. Effect of different level of lemon extract on quality and shelf life of chicken meatballs during frozen storage. SAARC Journal of Agriculture, 18(2): 139-156.

Gadang VP, Hettiarachchy NS, Johnson MG, Owens CM. 2008. Evaluation of antibacterial activity of whey protein isolate coating incorporated with nisin, grape seed extract, malic acid, and EDTA on a turkey frank further system. Journal of Food Science,73: 389-394.doi:10.1111/j.1750-3841.2008. 00899.x

Guerrero-Legarreta I. 2010. Handbook of poultry science and technology, Vol 2, Secondary processing. Mexico: willey. pp: 8-10.

Hara-Kudo Y, YamasakiA, Sasaki M, OkuboT, MinaiY, HagaM, Kondo K, Sugita-KonishiY. 2005. Antibacterial action on pathogenic bacterial spore by green tea catechins. J. Sci. Food Agric., 85:2354-2361.

Hossain MS, Rokib M, Habib M, Kabir MH, Hashem MA, Azad MAK, Rahman MM, Ali MS. 2021. Quality of spent hen sausages incorporated with fresh ginger extract. Meat Research, 1: 1, Article 4. https://doi.org/10.55002/mr.1.1.4

Huiyun Zhang, Jingjuan Wu, Xinyu Guo. 2015. Effects of antimicrobial and antioxidant activities of spice extracts on raw chicken meat quality. Food Science and Human Wellness, Volume 5, Issue 1, Pages 39-48.

Islam MA, Hossain MA,Sadakuzzaman M, Khan M, Rahman MM, Hashem MA. 2021. Effect of gamma irradiation on the shelf life and quality of mutton. Turkish Journalof Agriculture-Food Science and Technology, 10 (2): 117-124.

Jasim MT, Raauf MRA, Hana BD. 2011. The Antibacterial Activity of Callemia Sinensis (green tea) on Some Bacterial Species. College of Pharmacy, University of Mustansiriya. AJPS, Vol. 10, No.2.

Kristanti RA and Punbusayakul N. 2009. Antioxidant and antimicrobial activity of commercial green tea in Chiang Rai, Acta Horticultura pp: 837.

Mitsumoto M, O'Grady MN, Kerry JP, Joe Buckley D. 2005. Addition of tea catechins and vitamin C on sensory evaluation, colour and lipid stability during chilled storage in cooked or raw beef and chicken patties. Meat Science, 69:773–779.

Pajohi MR, Tajik H, Farshid AA, Basti AA, Hadian M. 2011. Effects of Mentha longifolia L. essential oil and nisin alone and in combination on Bacillus cereus and Bacillus subtilis in a food model and bacterial ultra-structural changes. Foodborne Pathog. Dis., 8, 283–290.

Patsias AV, Badeka IN, Savvaidis MG, Kontominasl. 2008. Combined effect of freeze chilling and MAP on quality parameters of raw chicken fillets, Food Microbiology, 25: 575–581.

Radji M, Agustama RA, Elya B, Tjampakasari CR. 2006. Antimicrobial activity of green tea extract against isolates of methicillin-resistant *Staphylococcus aureus* and multi-drug resistant *Pseudomonas aeruginosa*. J Am Coll Nutr., 25(2):79-99.

Saba NA, Hashem MA, Azad MAK, Hossain MA, Khan M. 2018. Effect of bottle gourd leaf (*Lagenariasiceraria*) extract on the quality of beef meatball. Bangladesh Journal of Animal Science, 47(2): 105-113.

Sadakuzzaman M, Hossain MA, Rahman MM, Azad MAK, Hossain MM, Ali MS, Hashem MA. 2021. Combined effect of irradiation and butylated hydroxyanisole on shelf life and quality of beef at ambient temperature. Meat Research, 1: 1, Article 3. https://doi.org/10.55002/mr.1.1.3

Sarah H, Hadiseh K, Gholam HA, Bahareh S. 2010. Effect of green tea (Camellia sinenses) extract and onion (Allium cepa) juice on lipid degradation and sensory acceptance of Persian sturgeon (Acipenserpersicus) fillet. International Food Research Journal, 17: 751-761.

Siddiqua T, Hossain MA, Khan M, Hashem MA. 2018. Formulation of value-added beef meatball using tulsi (Ocimum sanctum) leaf extract as a source of natural antioxidant. Journal of Bangladesh Agricultural University, 16(2): 260-265.

Sivarooban T, Hettiarachchy NS, Johnson MG. 2008. Transmission electron microscopy study of Listeria monocytogenes treated with nisin in combination with either grape seed or green tea extract. J. Food Prot., 71:2105–2109.

Su P, Henriksson A, Nilsson C, Mitchell H. 2008. Synergistic effect of green tea extract and probiotics on the pathogenic bacteria, *Staphylococcus aureus* and *Streptococcus pyogenes*. World Journal of Microbiology and Biotechnology, 24(9):837–1842.

Tang SZ, Kerry JP, Sheehan D, Buckley DJ, Morrissey PA. 2001. Antioxidative effect of added tea catechins on susceptibility of cooked red meat, poultry and fish to lipid oxidation. Food Research International, 34: 651-57.

Toda M, Okubo S, Hara Y, Shinamura T. 1991. Antibacterial and bactericidal activities of tea extracts and catechins against methicillin-resistant *Staphylococcus aureus*. Japanese Journal of Bacteriology, 46:839-845.

Wang SM, Zhao JF. 1997. Antioxidant activities of tea polyphenol on edible oil. Western Cereal Oil Technology, 22:44–46.

Zhu YX, Huang H,d Tu YY. 2005. A review of recent studies in China on the possible beneficial health effects of tea. International Journal of Food Science and Technology, 41:333–340.