The Pharma Innovation Journal 2022; SP-11(11): 2645-2648

www.ThePharmaJournal.com

The Pharma Innovation

**ISSN (E): 2277-7695**
**ISSN (P): 2349-8242**
**NAAS Rating: 5.23**
TPI 2022; SP-11(11): 2645-2648

www.thepharmajournal.com
Received: 12-09-2022
Accepted: 19-10-2022

**Solanki Bhoomi A**
Bhartiya Polytechnic in Animal, Husbandry, Kamdhenu University, Surat, Gujarat, India

**Roy SK**
Department of Livestock Products Technology, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada, Gujarat, India

**Prajapati Bhavesh I**
Department of Veterinary Public, Health and Epidemiology, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar, Dantiwada, Gujarat, India

**Goswami Mayank M**
Noble Polytechnic in Animal, Husbandry, Kamdhenu, University, Junagadh, Gujarat, India

**Corresponding Author:**
**Solanki Bhoomi A**
Bhartiya Polytechnic in Animal, Husbandry, Kamdhenu University, Surat, Gujarat, India

# Studies on augmentation of shelf-life of meat using spices at refrigeration temperature

**Solanki Bhoomi A, Roy SK, Prajapati Bhavesh I and Goswami Mayank M**

**Abstract**
The present study was conducted to evaluate antimicrobial effect of garlic, cinnamon, clove and nitrite in complex food system like mince chicken stored at refrigeration temperature. It was an attempt to replace nitrite with spices in minced chicken. Antimicrobial studies (*Escherichia coli*, *Staphylococcus aureus* and *Clostridium perfringens*) were carried out on 1, 4, 7 and 10$^{th}$ day of storage. Garlic paste (3 per cent), Cinnamon powder (2 per cent w/w) and Clove powder (0.2 per cent w/w) were significantly effective ($p \leq 0.05$) against *Escherichia coli* and *Staphylococcus aureus* but slightly lower effect against *Clostridium perfringens*. Antimicrobial activity of clove powder was higher against *S. aureus, E.coli* and *C. perfringenes* count as compare to control. Clove powder could be used as nitrite replacer however not effective as nitrite.

**Keywords:** Mince chicken, natural preservatives, nitrite, *Clostridium perfringens*, *Escherichia coli*, *Staphylococcus aureus*

## 1. Introduction
Meat is highly nutritious food for *Homo sapiens* since time immemorial. It provides high quality protein, important minerals, vitamins et cetera. It is nutrient compact food. In India, poultry industry has made impressive progress during the last three decades evolving from backyard venture to a full-fledged commercial agro industrial business. Hence, there is a great concern for the quality and safety of processed poultry foods being offered. Today's consumers demand foods with high nutritional value that are free from chemical preservatives and are microbiologically safe (Kumar *et al*., 2021; Goswami *et al*., 2020) [13, 7]. The activity of nitrite is in broad spectrum so it is not possible aid and so combinations of antimicrobials are used (Sofos, 2008) [21]. There has been a constant search for alternative and efficient compounds for meat preservation, aiming at partial or total replacement of nitrite in cured meat and meat products. Microbial control in foods can be assured by suppressing one or more factors essential for microbial survival (Horace, 1982) [10] and here many spices offer a promising alternative for ensuring food safety. Small concentrations of nitrite are sufficient to cause a broad spectrum inhibition of *Clostridium Botulinum*.
Spices and herbs have been used for thousands of centuries by many nations to enhance the flavour and aroma of foods and has documented in the late 19th century and they are having antimicrobial properties. Spices like Garlic (*Allium sativum*) is a broad spectrum and called as dietary antibiotic and also having flavour enhancer properties in meat product, along with medicinal attributes. These include antibacterial, antiviral, antifungal and antiprotozoal activities (Ankari and Mirelman, 1999) [3]. Cloves (*Syzygium arromaticum*) are dried bud of this tree, which contain 14% - 21% essential oil and main active ingredient of clove is euganol, which have potent antimicrobial activity and keeps the food fresh. As reported in literature the combination of both clove and its extract doubled the shelf life of minced chicken meat (Yadav and Singh 2004) [19] Clove extract is also inhibit the growth of *Salmonella typhinurium* (Singh *et al*., 2004) [19]. Cinnamon (*Cinnamomum zylancium*) one of the most common spices and food flavoring additives since ancient times (Wijesekera *et al*., 1997) [23], shows that cinnamon has potent antiemetic, anti-diarrheal, anti-flatulent and stimulant activities. (Hossein *et al*., 2013) [11]. Recent, scientific reports showed that cinnamon has potent neuroprotective, hepatoprotective, cardioprotective and gastroprotective effects due to its potent antioxidant and anti-inflammatory properties (Khasnavis *et al*., 2012; Alqasoumi *et al*., 2011) [12, 1]. Apart from this it has antimicrobial properties for both Gram-positive and Gram- negative bacteria.

Microbiologist have been testing effectiveness of cinnamon and other spices in eliminating most virulent bacteria causes of food poisoning *Escherichia .coli* O157. In nutshell it can be said that spices like garlic, clove and cinnamon has the ability to stop bacterial growth. Therefore, on the basis of this postulation, a study was undertaken.

## 2. Materials and Methods

### 2.1 Material Procurement

Fresh broiler chicken meat required for the experiments was procured from the meat shop located at Palanpur, Gujarat. India. Cinnamon, Clove and Garlic was procured from local market. Nitrite used in the study was of analytical grade and purchased from S.D fines chem. Ltd, Mumbai. Low Density Polyethylene (LDPE-0.0035mm) was procured from market and sterilized by exposing to U.V light for 30 minutes before use. *Staphylococcus aureus* and *Escharichia coli* cultured was procured from department of Veterinary Public Health and Epidemiology of College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar while *Clostridium perfringens* (MTCC 450) procured from Microbial Type Culture Collection center, Institute of Microbial Technology, Chandigarh, India.

### 2.2 Preparation of Stock Inoculum

The standard cultures of *Clostridium perfringens* (MTCC 450); *Escharichia coli* and *Staphylococcus aureus* were revived under the aseptic conditions as per the instructions.

### 2.3 Preparation of Meat Mince

The boneless chicken meat was wash thoroughly. All the food contact surfaces of meat mincer (made up of stainless steel contact parts) were sanitized before mincing. The Deboned meat was cut into small cubes and minced in Stadler meat grinder using 8 mm plates.

### 2.4 Preparation of clove, cinnamon powder and garlic paste

Clove powder and Cinnamon powder were obtained by grinding the good quality cloves and cinnamon in a grinder and sieved through a fine mesh. The fine powders were stored in a jar for subsequent use. Fresh garlic paste was prepared after washing, peeling and blending of garlic.

### 2.5 Portioning of meat mince and incorporation of Garlic, Cinnamon, Clove and Nitrite

Minced meat was divided into approximately five equal portions and assigned to following treatments: Control (C); Sodium Nitrite – 200ppm (N); Garlic paste – 3 per cent ($T_1$); Cinnamon powder – 2 per cent w/w ($T_2$); Clove powder – 0.2 per cent w/w ($T_3$)Each portion was weighed accurately and recorded. Then they were incorporated thoroughly by kneading or massaging for uniform distribution. Each of treatments C, N, $T_1$, $T_2$, and $T_3$ portions were further sub divided into two parts.

### 2.6 Incorporation of bacterial inoculums in the minced chicken

The broth containing known concentration of *Clostridium perfringens*, *Escharichia* and *S. aureus* stored in refrigerator were serially diluted separately with the normal saline solution to approximately $10^7$ bacterial colonies per ml. 1ml of each inoculum was added to100g of the minced chicken (taken in sterilized LDPE bags) separately and then thoroughly mixed the kneding, so that final concentration of approximately $10^5$ cfu/g of the meat mince could be obtained. The concentrations of bacterial cultures were kept deliberately high to minimize the sampling error.

### 2.7 Packaging and Storage

The LDPE bags containing inoculated chicken mince were sealed with the help of a sealer (Sevana's India) and stored at refrigeration temperature ($4\pm1^0$C) till further analysis. The antimicrobial (APHA, 1984) studies were carried out on 1, 4, 7 and 10$^{th}$ day of storage. A total of 6 replications were carried out with each analysis done in duplicate.

### 2.8 Statistical analysis

The data obtained were analyzed by using ANOVA technique by Snedecor and Snedecor and Cochran (1989) [20].

## 3. Result and Discussion

Effect of Garlic, Cinnamon, Clove and nitrite treated minced chicken on microbiological quality in table.

### 3.1 *Escharichia coli* count

The *Escharichia coli* count of control and treated samples increased over storage periods. Means of *Escharichia coli* count (log cfu/g) were significantly lower ($p<0.01$) for $T_1$, $T_2$, $T_3$, compared to control. The *Escharichia coli* count (log cfu/g) of C samples were 5.906±0.008, 6.806±0.01, 8.395±0.003 and 9.403±0.008 in 1, 4, 7and 10$^{th}$ storage day respectively. Morita and Hiroshi (2004) [14] in their study explained that the antibacterial activity of NO (Nitric oxide) derived from sodium nitrite against *Escharichia coli* count 0157:H7 was due to generation of dinitrosyl Fe complex from the reaction of Fe-S –protein (enzymes) with NO (Nitric oxide). Enzymes related to respiratory chain were inactivated resulting in decreased ATP levels for cells. Similar results were reported by Neogi *et al.* (2007) [15]; Friedman *et al.* (2002) [5]; Seema *et al.* (2007) [17] and Gupta *et al.* (2008) [8].

**Table 1:** *Escharichia coli* count (log cfu/g) of Garlic, cinnamon, clove and nitrite treated minced chicken for different treatments and storage period

| Storage days | Control (C) | 3%Garlic paste ($T_1$) | 2% w/w Cinnamon powder($T_2$) | 0.2% w/w Clove powder ($T_3$) | 200ppm Nitrite (N) |
|---|---|---|---|---|---|
| 1 | $5.906\pm0.008^{ap}$ | $5.776\pm0.01^{bp}$ | $5.796\pm0.01^{cp}$ | $5.762\pm0.01^{dp}$ | $5.658\pm0.01^{ep}$ |
| 4 | $6.806\pm0.01^{aq}$ | $6.169\pm0.008^{bq}$ | $6.234\pm0.01^{cq}$ | $6.166\pm0.009^{dq}$ | $5.940\pm0.01^{eq}$ |
| 7 | $8.395\pm0.003^{ar}$ | $6.331\pm0.01^{br}$ | $6.371\pm0.01^{cr}$ | $6.359\pm0.006^{dr}$ | $6.273\pm0.01^{er}$ |
| 10 | $9.403\pm0.008^{as}$ | $8.234\pm0.01^{bs}$ | $8.202\pm0.007^{cs}$ | $8.196\pm0.007^{ds}$ | $8.095\pm0.01^{es}$ |

Mean ± S.E. with different superscripts between rows (p, q, r, s) and between columns (a, b, c, d) differ significantly ($P < 0.05$).

### 3.2 *Stapylococcus aureus* count

The Table. 2 shows that control samples showed mean count (log cfu/g) 6.275±0.005 on 10$^{th}$ days of storage. The *Stapylococcus aureus* count (log cfu/g) for treated samples showed the similar trend. The *Stapylococcus aureus* count of control and treated samples increased over storage periods.

Highly Significant reduction (P<0.01) in *Stapylococcus aureus* count was observed between $T_1$, $T_2$, $T_3$, N compared to control. Treated samples showed lower count compare to control but slightly higher than the nitrite it may be due the most important active substance i.e. cinamicaldehyde and eugenol. Similar results were given by Seema *et al.* (2007) [17]; Sharma *et al.* (2012) [18]; Gupta *et al.* (2008) [8]; Pandey *et al.* (2014) [16].

**Table 2:** *Stapylococcus aureus* count (log cfu/g) of garlic, cinnamon, clove and nitrite treated minced chicken for different treatments and storage period

| Storage days | Control (C) | 3%Garlic paste ($T_1$) | 2% w/w Cinnamon powder($T_2$) | 0.2% w/w Clove powder ($T_3$) | 200ppm Nitrite (N) |
|---|---|---|---|---|---|
| 1 | $5.884\pm0.01^{ap}$ | $5.686\pm0.01^{bp}$ | $5.712\pm0.02^{cp}$ | $5.682\pm0.02^{dp}$ | $5.532\pm0.02^{ep}$ |
| 4 | $5.910\pm0.006^{ap}$ | $5.893\pm0.01^{aq}$ | $5.834\pm0.01^{bq}$ | $5.852\pm0.009^{cq}$ | $5.759\pm0.02^{dq}$ |
| 7 | $6.179\pm0.008^{aq}$ | $5.982\pm0.01^{br}$ | $6.006\pm0.01^{cr}$ | $5.923\pm0.01^{dr}$ | $5.860\pm0.01^{er}$ |
| 10 | $6.275\pm0.005^{ar}$ | $6.049\pm0.01^{bs}$ | $6.184\pm0.008^{cs}$ | $6.023\pm0.02^{ds}$ | $5.985\pm0.01^{es}$ |

Mean ± S.E. with different superscripts between rows (p, q, r, s) and between columns (a, b, c, d) differ Significantly (P < 0.05).

### 3.3 *Clostridium perfrigens* count

The *Clostridium perfrigens* count (table 3) of control and treated samples increased over storage periods. Treatments mean of *Clostridium perfrigens* count for $T_1$, $T_2$, $T_3$ and N were significantly lower (p<0.01) as compared to control.

**Table 3:** *Clostridium perfrigens* count (log cfu/g) of garlic, cinnamon, clove and nitrite treated minced chicken for different treatments and storage period

| Storage days | Control (C) | 3%Garlic paste ($T_1$) | 2% w/w Cinnamon powder($T_2$) | 0.2% w/w Clove powder ($T_3$) | 200ppm Nitrite (N) |
|---|---|---|---|---|---|
| 1 | $6.233\pm0.01^{ap}$ | $5.201\pm0.01^{bp}$ | $5.648\pm0.01^{cp}$ | $5.723\pm0.01^{dp}$ | $5.187\pm0.008^{ep}$ |
| 4 | $8.474\pm0.007^{aq}$ | $7.100\pm0.01^{bq}$ | $7.300\pm0.007^{cq}$ | $6.194\pm0.005^{dq}$ | $6.245\pm0.01^{eq}$ |
| 7 | $9.410\pm0.009^{ar}$ | $9.177\pm0.01^{br}$ | $9.297\pm0.005^{cr}$ | $9.119\pm0.007^{dr}$ | $7.365\pm0.005^{er}$ |
| 10 | $9.969\pm0.01^{as}$ | $9.361\pm0.003^{bs}$ | $9.349\pm0.003^{cs}$ | $9.330\pm0.005^{ds}$ | $8.200\pm0.01^{es}$ |

Mean ± S.E. with different superscripts between rows (p, q, r, s) and between columns (a, b, c, d) differ significantly (P < 0.05).

The *Clostridium perfringens* count of control and treated samples increased over storage periods. The mean *Clostridium perfringens* count (log CFU/g) were significantly lower (p<0.05) for $T_1$ (3% garlic paste), $T_2$ (2% w/w cinnamon powder, $T_3$ (0.2% clove powder), and N (Nitrite) when it is compared to Control. Nitrite was found most effective among all treatments. The findings were in accordance with Amin and Oliviera (2006) [22] in sausages; Taormina *et al.* (2003) [22] in that processed meat products. Hernández-Ochoa *et al.* (2011) [9] also examine minimum inhibitory concentration of essential oil of clove and cumin against *Escherichia coli* O157:H7, *Salmonella, Listeria monocytogenes, Yersinia enterocolitica, Campylobacter jejuni, Clostridium perfringens, Stphylococcus aureus* and *Toxplasma Gondi*. Clove showed a reduction of 3.78 log CFU/g with application of 2,250µL oil; Goswami *et al.* (2014) [6] also reported reduce *Clostridium perfringens* using spices at refrigeration; Sharma *et al.* (2012) [18] in chicken mince using turmeric.

## 4. Conclusion

The present study was made as an attempt to compare the antimicrobial properties spices like Garlic, Cinnamon and Clove with nitrite. $T_1$ (3% garlic paste), $T_2$ (2%w/w cinnamon powder) and $T_3$ (0.2% w/w clove powder) were significantly effective (p≤0.05) against *Escharichia coli*. Clove, Cinnamon powder, Garlic paste having good antimicrobial activity than other spices against food pathogenic bacteria. Nitrite was most effective against all treated samples followed by $T_3$, $T_1$ and $T_2$ in chicken mince. These findings revealed that spices having noble antimicrobial activity which could be replaced for preservation of meat and meat products but further research work in this direrction is required to confirm in-vivo and *in-vitro* in future.

## 5. Acknowledgment

The authors are highly grateful to acknowledge the support rendered by the Dean, college of Veterinary Science and A.H. and Director of Research, Sardar Krushinagar Dantiwada Agricultural University for financial assistance and research facilities.

## 6. References

1. Alqasoumi S, Al-Dosary M, Al-Yahya M, Al-Mofleh I. Gastroprotective effect of a popular spice cinnamon *Cinnamomum zeylanicum* in rats. European Journal of Pharmacology. 2011;668(1):42.
2. Amin M, Oliviera, JV. The effect of the use nitrite and nitrate on the inhibition of *Clostridium perfringens* type A on the bovine cured sausages. Boletim do Centro de Pesquisa de Processamento de Alimento. 2006;24(1):13-24.
3. Ankari S, Mirelman D. Antimicrobial properties of allicin from garlic. Microbes and infection. 1999;2:125-129.
4. APHA. Compendium of methods for the microbiological examination of foods. 4th (ed.) Andergrant, C. V. and Plittstoesser, D. F. S. American Public Health Association, Washington, D.C; c1984. p. 919-927.
5. Friedman M, Henika PR, Mandrell RE. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica*. Journal of Food Protection. 2002;65:1545-1560.
6. Goswami M, Prbhakaran P, Tanvar VK. Antioxidant and antimicrobial effects of Condiments paste used as nitrite replacer in chicken mince. Veterinary World. 2014;7(6):432-438.
7. Goswami MM, Roy SK, Prajapati BI, Deokar SS, Nalwaya SB, Solanki BA. Effect of gooseberry pulp and seed coat powder as natural preservatives on the storage quality of chicken nuggets. Journal of Animal Research. 2020;10(4):601-607.
8. Gupta C, Garg AP, Uniyal RC, Kumari A. Comparative

analysis of the antimicrobial activity of cinnamon oil and cinnamon extract on some food-borne microbes. African Journal of Microbiology Research. 2008;2(9):247-251.
9. Hernández-Ochoa L, Aguirre-Prieto YBG, Nevárez-Moorillón V, Gutierrez-Mendez N, Salas-Muñoz E. Use of essential oils and extracts from spices in meat protection. Journal of Food Science and Technology. 2011;51(5):957-963.
10. Horace DG. The safety of foods. Cited in: Goswami M, Prabhakaran P, Tanwar VK, Comparative study of spices and condiments mix as nitrite replacer for reducing microbial load in chicken mince. Journal of Meat science and Technology. 2013;1(3):91-97.
11. Hossein N, Zahra Z, Abolfazl M, Mahdi S, Ali K. Effect of Cinnamon zeylanicum essence and distillate on the clotting time. Journal of medicinal plant research. 2013;7:1339-1343.
12. Khasnavis S, Pahan K. Sodium benzoate, a metabolite of cinnamon and a food additive, upregulates neuroprotective Parkinson disease protein DJ-1 in astrocytes and neurons. Journal of Neuroimmune Pharmacology. 2012;7:424–435.
13. Kumar P, Kaur S, Goswami M, Singh S, Sharma A, Mehta N. Antioxidant and antimicrobial efficacy of giloy (*Tinospora cordifolia*) stem powder in spent hen meat patties under aerobic packaging at refrigeration temperature (4±1°C). Journal of Food Processing and Preservation. 2021;45(10):e15772.
14. Morita H, Hiroshi Y, Antimicrobial action against verotoxigenic *Escherichia coli* O157:H7 of nitrite oxide derived from sodium nitrite. BioScience- Biotechnology and Biochemistry. 2004;68(5):1027-1034.
15. Neogi U, Saumya R, Irum B. *In vitro* Combinational Effect of Bio-Active Plant Extracts on Common Food Borne Pathogens. Research Journal of Microbiology. 2007;2(5):500-503.
16. Pandey B, Khan S, Singh S. A Study of antimicrobial activity of some spices. International Journal of Current Microbiology and Applied Sciences. 2014;3(3):643-650.
17. Seema A, Dostbil N, Alemdar S. Antimicrobial effect of some spices used in meat industry. Bulletin of the Veterinary Institute in Pulawy. 2007;51:53-57.
18. Sharma J, Prabhakaram PP, Tamwar VK. Antioxidant and antimicrobial effect of turmeric and nitrite on mince chicken. Journal of Animal research. 2012;2(3):299-306.
19. Singh I, Yadav AS, Pandey NK, Singh RP. Antimicrobial effect of extract of spice against *Sallmonella typhimurium* in chicken patties during refrigeration storage under vacuum packaging. In: Proceedings XXII World's Poultry Congress, Istambul, Turkey. 2004;13:857-859.
20. Snedecor GW, Cochran WG. Statistical methods. 8th ed. IOWA state university press, Ames, IOWA; c1989.
21. Sofos JN, Challenges to meat safety in the 21st century. Meat Science. 2008;78:3-13.
22. Taormina PJ, Bartholomew GW, Dorsa WJ. (Incidence of *Clostridium perfringnes* in commercially produced cured raw meat product mixtures and behaviour in cooked products during chilling and refrigerated storages. Journal of Food Protection. 2003;66 (1):72-81.
23. Wijesekera R. Historical overview of the cinnamon industry. Critical Reviews in Food Science and Nutrition. 1997;10:1-30.
24. Yadav AS, Singh RP. Natural preservative in poultry meat products. Natural Products Radiance. 2004;3(4):300-303.