

Antimicrobial and Antioxidant Activity of Spices (Garlic, Cinnamon, Clove) in Minced Chicken at Refrigeration Storage

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ABSTRACT

The present study was conducted to evaluate antioxidant and antimicrobial effect of garlic, cinnamon and clove as nitrite replacer in chicken mince meat stored at refrigeration temperature ($4\pm 1^\circ\text{C}$). Chemical characteristics (pH, TBARS) were assessed on 0, 3, 6 and 9th day of storage, while antimicrobial studies (*Escherichia coli*, *Staphylococcus aureus* and *Clostridium perfringens*) were carried out on 1, 4, 7 and 10th day. The pH values increased gradually and significantly ($p < 0.05$) over each storage interval in all treatments and control; it was highest in T3 (0.2% clove powder) and lowest in T2 (2% w/w cinnamon powder) or T1 (3% garlic paste), while in control and N treated samples it was intermediate. TBA values were significantly ($p < 0.05$) lower in T1 followed by N, T2 and T3, and highest in control samples at all storage intervals, and increased significantly with each storage interval from day 0 to 9. Inclusion of Garlic paste (3%), Cinnamon powder (2% w/w) and Clove powder (0.2% w/w) in chicken mince meat was significantly effective ($p \leq 0.05$) against *Escherichia coli* and *Staphylococcus aureus*, but had slightly lower effect against *Clostridium perfringens*. In conclusion, clove having good antioxidant activity in complex food system like chicken mince where nitrite can be replaced with clove. Antimicrobial activity of clove powder was higher against *S. aureus*, *E. coli* and *C. perfringens* count as compared to control.

Key words: Chicken, *Clostridium perfringens*, *Escherichia coli*, Natural preservatives, Nitrite, *Staphylococcus aureus*.

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INTRODUCTION

Meat is highly nutritious food for humans since time immemorial. It is nutrient compact food, which provides high quality protein, important minerals, vitamins etc. In India, poultry industry has made awesome growth in 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. Poultry meat is an ideal medium for growth of microbes. Today's consumers demand foods with high nutritional value that are free from chemical preservatives and are microbiologically safe. In curing salt, nitrates, nitrites, sugar and their combination are added in the product with the aim of drawing moisture out of the food by the process of osmosis. Nitrite is having antibotulinal, antioxidant and antimicrobial properties. It also contributes flavour and colour due to formation of nitric oxide myochrome [(NO)₂MC] in cooked meat (Douglas *et al.*, 1975). Regardless of the technological benefits, a reduction in the use of nitrites has become a key issue for the industry. This is because nitrite can under certain circumstances (low pH and high temperature) react with amines to form nitrosamines, compounds that have shown in variety of animal studies to be carcinogenic (Jakszyn and Gonzalez, 2006). Therefore, the reduction of residual nitrite or its removal in meat can reduce the exposure of people to nitrosamines and thereby reduce the total exposure to carcinogens. But all functions performed by nitrite in processed meat, the antimicrobial

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effect including activity against *Clostridium botulin* is the single most important reason cited by meat industry to not to eliminate nitrite from meat curing while it's level is strongly restricted to 200 ppm (FSSAI, 2011).

One method to avoid the direct addition of nitrite to meat is adding ingredients that have natural high nitrate content. This method is used in the production of organic versions of cured meats (Sebranek and Bacus, 2007). Another alternative

of nitrite is to add naturally occurring antimicrobials to meat products where they synthetically-derived antimicrobials compounds that may be present in the spices, herbs or their essential oils including terpenes, coumarins, and flavonoids (Kim *et al.*, 1995) can play an important role. Other naturally occurring antimicrobials are nisin (microbial source), lysozyme, and antimicrobial polypeptides (AMPs) (animal source) (Gaysinsky and Weiss, 2007). 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, and here many spices offer a promising alternative for ensuring food safety.

Spices like Garlic (*Allium sativum*) have been used since ancient times for both medicinal as well as culinary purpose; it is a broad spectrum and called as dietary antibiotic. It has antibacterial, antiviral, antifungal and antiprotozoal activities (Ankri and Mirelman, 1999) and it also has beneficial effects on the cardiovascular and immune system. Cloves (*Syzygium aromaticum*) are dried bud of this tree. The main active ingredient of clove is eugenol, which in addition to kill bacteria and viruses keeps the food fresh. Incorporation of alcoholic clove extract at 0.15% w/w level alone or combination with *Lactobacillus acidophilus* (LA culture) in minced chicken meat exhibited potent antimicrobial and antioxidant effect during storage at refrigeration temperature. As reported in literature the combination of both clove and its extract doubled the shelf life of minced chicken meat (Yadav and Singh, 2004). Cinnamon (*Cinnamomum zylancium*) has potent antiemetic, anti-diarrheal, anti-flatulent and stimulant activity (Hossein *et al.*, 2013) in addition to potent broad spectrum antibacterial, antifungal, anti-termite, larvicidal, nematicidal, and insecticidal properties (Cheng *et al.*, 2004). In nutshell, it can be said that spices like garlic, clove and cinnamon has the ability to stop bacterial growth, has anti-oxidative properties and hence these compounds replaced the nitrite in meat product. Therefore, on the basis of this postulation, this study was undertaken to evaluate the antimicrobial and antioxidant activity of these spices in chicken mince meat.

MATERIAL AND METHODS

Material Procurement

Fresh broiler chicken meat required for the experiment was procured from the meat shop located at Palanpur, Gujarat, India. Cinnamon, Clove and Garlic were 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-0.0035 mm) bags were procured from market and sterilized by exposing to UV light for 30 min before use. *Staphylococcus aureus* and *Escherichia coli* cultures was procured from Department of Veterinary Public Health and Epidemiology of Veterinary College, Sardarkrushinagar-Dantiwada Agricultural University, Sardarkrushinagar, while *Clostridium perfringens* (MTCC 450) procured from Microbial

Type Culture Collection centre, Institute of Microbial Technology, Chandigarh, India.

Preparation of Stock Inoculum and Meat Mince

The standard cultures of *Clostridium perfringens* (MTCC 450), *Escherichia coli* and *Staphylococcus aureus* were revived under the aseptic conditions as per the instructions. The boneless chicken meat was washed 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.

Preparation of Clove, Cinnamon Powder and Garlic Paste

Clove and Cinnamon powders 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 jar for subsequent use. Fresh garlic paste was prepared after washing, peeling and blending of garlic.

Partitioning 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 - 200 ppm (N); Garlic paste - 3 % w/w (T1); Cinnamon powder - 2 % w/w (T2); and Clove powder - 0.2 % w/w (T3). Each portion was weighed accurately, recorded and incorporated thoroughly by kneading or massaging for uniform distribution. Each of treatments C, N, T1, T2, and T3 were further subdivided into two parts. One part used to check antioxidant and chemical properties, while another part used for microbiological studies.

The portion of minced chicken intended for the microbiological studies was subdivided accurately into 100 g portions for inoculation with standard cultures of *Clostridium perfringens*, *Escherichia coli* and *Staphylococcus aureus*.

Incorporation of Bacterial Inoculums in the Minced Chicken

The broth containing known concentration of *Clostridium perfringens*, *Escherichia coli* and *S. aureus* stored in refrigerator were serially diluted separately with the normal saline solution to approximately 10^7 cfu/mL. 1 mL of each inoculum was added to 100 g of the minced chicken (taken in sterilized LDPE bags) separately and then thoroughly mixed by kneading, 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.

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 ($4\pm 1^\circ\text{C}$) till further analysis. The

mince portions assigned for studies on antioxidative and chemical properties were also sealed in LDPE bags and stored at refrigeration temperature ($4\pm 1^\circ\text{C}$) as above till further analysis. The chemical and antioxidative studies were carried out on 0, 3, 6, and 9th day of storage and antimicrobial studies were carried out on 1, 4, 7 and 10th day of storage. A total of 6 replications were used with each analysis done in duplicate.

The pH of meat samples was determined as per the method of Trout *et al.* (1992). For thiobarbituric acid (TBA) value the thiobarbituric reacting substances (TBARS) method of Witte *et al.* (1970) was followed.

Statistical Analysis

The data obtained were analyzed by using ANOVA technique and Duncan's multiple range test as per Snedecor and Cochran (1994) to know the statistical significance at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of Garlic, Cinnamon, Clove and Nitrite treated minced chicken on microbiological and biochemical quality during refrigeration storage till 10 days are shown in Table 1 and 2, respectively.

Escherichia coli Count

The *Escherichia coli* count of control and treated samples increased gradually and significantly ($p < 0.05$) over each storage interval till 10th day. Means of *Escherichia coli* count (log cfu/g) were significantly lower ($p < 0.01$) for T1, T2, T3 and N compared to control. The values in samples treated with 200 ppm nitrite (N) were significantly lower followed by 0.2 % w/w clove powder (T3), and 3% garlic paste (T1), and values for 2% w/w cinnamon powder (T2) were mostly intermediate of T1 and T3 at most storage intervals (Table 1). Morita and Hiroshi (2004) in their study explained that the antibacterial activity of NO (Nitric oxide) derived from sodium nitrite against *Escherichia coli* count 0157:H7

was due to generation of dinitrosyl Fe complex from the reaction of Fe-S-protein (enzymes) with NO. Enzymes related to respiratory chain were inactivated resulting in decreased ATP levels for cells. Similar results were reported by Friedman *et al.* (2002), Seema *et al.* (2007) and Gupta *et al.* (2008).

Staphylococcus aureus Count

The control samples showed mean *Staphylococcus aureus* count (log cfu/g) of 5.884 ± 0.01 , 5.910 ± 0.006 , 6.179 ± 0.008 and 6.275 ± 0.005 on 1, 4, 7 and 10th day of refrigeration storage, respectively, being increased gradually and significantly with advancing storage time (Table 1). The *Staphylococcus aureus* count (log cfu/g) for all treatments also showed the similar trend, and increased over storage periods. However, highly significant reduction ($p < 0.01$) in *S. aureus* counts was observed in N, T1, T2, and T3 compared to control at all intervals. The herbal treated samples showed significantly lower counts compared to control and higher counts than the nitrite. The decreased *Staphylococcus aureus* count in cinnamon and clove compared to control may be due their most important active substances, *i.e.* cinamaldehyde and eugenol. Similar results were reported by Seema *et al.* (2007); Gupta *et al.* (2008) and Sharma *et al.* (2012).

Clostridium perfringens Count

The *Clostridium perfringens* count (Table 1) of control and treated samples also increased gradually and significantly ($p < 0.05$) over each storage interval till 10th day. The mean *C. perfringens* counts (log CFU/g) were significantly lower ($p < 0.05$) for T1 (3% garlic paste), T2 (2% w/w cinnamon powder, T3 (0.2% clove powder), and N (Nitrite) when compared with control. Nitrite was found most effective among all treatments, while the *C. perfringens* counts in three herbal treatments varied inconsistently among themselves over different storage periods. These findings

Table 1: Mean (\pm SE, n=6) counts of *Escherichia coli*, *Staphylococcus aureus* and *Clostridium perfringens* (log cfu/g) in garlic, cinnamon, clove and nitrite treated and control minced chicken at different storage periods ($4\pm 1^\circ\text{C}$)

Organism	Storage Days	Control (C)	3% Garlic paste (T1)	2% w/w Cinnamon powder (T2)	0.2% w/w Clove powder (T3)	200 ppm Nitrite (N)
<i>Escherichia coli</i>	1	5.906 \pm 0.008 ^{as}	5.776 \pm 0.01 ^{cs}	5.796 \pm 0.01 ^{bs}	5.762 \pm 0.01 ^{ds}	5.658 \pm 0.01 ^{es}
	4	6.806 \pm 0.01 ^{ar}	6.169 \pm 0.008 ^{cr}	6.234 \pm 0.01 ^{br}	6.166 \pm 0.009 ^{cr}	5.940 \pm 0.01 ^{dr}
	7	8.395 \pm 0.003 ^{aq}	6.331 \pm 0.01 ^{dq}	6.371 \pm 0.01 ^{bq}	6.359 \pm 0.006 ^{cq}	6.273 \pm 0.01 ^{eq}
	10	9.403 \pm 0.008 ^{ap}	8.234 \pm 0.01 ^{bp}	8.202 \pm 0.007 ^{cp}	8.196 \pm 0.007 ^{cp}	8.095 \pm 0.01 ^{dp}
<i>Staphylococcus aureus</i>	1	5.884 \pm 0.01 ^{as}	5.686 \pm 0.01 ^{cs}	5.712 \pm 0.02 ^{bs}	5.682 \pm 0.02 ^{cs}	5.532 \pm 0.02 ^{ds}
	4	5.910 \pm 0.006 ^{ar}	5.893 \pm 0.01 ^{br}	5.834 \pm 0.01 ^{dr}	5.852 \pm 0.009 ^{cr}	5.759 \pm 0.02 ^{er}
	7	6.179 \pm 0.008 ^{aq}	5.982 \pm 0.01 ^{cq}	6.006 \pm 0.01 ^{bq}	5.923 \pm 0.01 ^{dq}	5.860 \pm 0.01 ^{eq}
	10	6.275 \pm 0.005 ^{ap}	6.049 \pm 0.01 ^{cp}	6.184 \pm 0.008 ^{bp}	6.023 \pm 0.02 ^{dp}	5.985 \pm 0.01 ^{ep}
<i>Clostridium perfringens</i>	1	6.233 \pm 0.01 ^{as}	5.201 \pm 0.01 ^{ds}	5.648 \pm 0.01 ^{cs}	5.723 \pm 0.01 ^{bs}	5.187 \pm 0.008 ^{es}
	4	8.474 \pm 0.007 ^{ar}	7.100 \pm 0.01 ^{cr}	7.300 \pm 0.007 ^{br}	6.194 \pm 0.005 ^{er}	6.245 \pm 0.01 ^{dr}
	7	9.410 \pm 0.009 ^{aq}	9.177 \pm 0.01 ^{cq}	9.297 \pm 0.005 ^{bq}	9.119 \pm 0.007 ^{dq}	7.365 \pm 0.005 ^{eq}
	10	9.969 \pm 0.01 ^{ap}	9.361 \pm 0.003 ^{bp}	9.349 \pm 0.003 ^{cp}	9.330 \pm 0.005 ^{dp}	8.200 \pm 0.01 ^{ep}

Mean \pm SE values with different superscripts between rows (p, q, r, s) and between columns (a, b, c, d) for an organism differ significantly ($p < 0.05$).



were in accordance with Amin and Oliviera (2006) in sausages and Taormina *et al.* (2003) in the processed meat products. Hernández-Ochoa *et al.* (2011) examined minimum inhibitory concentration of essential oil of clove and cumin against *Escherichia coli* O157:H7, *Salmonella*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Campylobacter jejuni*, *Clostridium perfringens*, *Staphylococcus aureus* and *Toxoplasma gondii*. Clove showed a reduction of 3.78 log CFU/g with application of 2,250 µL oil. Sharma *et al.* (2012) and Goswami *et al.* (2014) also reported reduced *Clostridium perfringens* counts in chicken mince using condiments spices and turmeric as nitrite source at refrigeration storage, respectively.

pH of Chicken Mince

The mean pH values of control and treatment samples of minced chicken on storage days 0, 3, 6, and 9th increased gradually and significantly ($p < 0.05$) over each storage interval (Table 2). It was highest in treatment T3 (0.2% clove powder) and lowest in T2 (2% w/w cinnamon powder) or T1 (3% garlic paste) at different time intervals, while the values in control and N treated samples had intermediate values. Addition of clove and cinnamon decreased the pH due to eugenol and cinnamaldehyde which stimulates the lactic acid, which in turn causes drop in microbial activity (Varel and Muller, 2004). The results of this study were in agreement with Yadav *et al.* (2005) in minced chicken during 10 days of refrigerated storage and Singh *et al.* (2005) in chicken meat treated with alcoholic extract of clove. Chicken meat treated with garlic paste also resulted in lowering pH compared to control or N treatment. This decreased pH may be attributed to differential inhibitory activity of garlic on bacteria; lactic acid bacteria being the least sensitive microorganism (Rees *et al.*, 1993). As the storage day advanced pH was increased in all samples, such an increase in pH reflects degree of meat spoilage through protein breakdown for production of free amino acids leading to the formation of NH and amines, compounds of alkaline reaction (Karabagis *et al.*, 2011).

Thiobarbituric Acid (TBA) Values (mg Malonaldehyde/kg)

The TBA value of all treated (T1, T2, T3 and N) and control samples increased significantly ($p \leq 0.05$) as the storage days advanced from day 0 to 9. The garlic, cinnamon, clove and nitrite added chicken mince showed significantly lower TBARS values as compared to control (Table 2). The lowest TBA values were recorded at all storage intervals in treatment T1 (3% Garlic paste) followed by N (200 ppm Nitrite), T2 (2 % w/w Cinnamon powder) and T3 (0.2 % w/w Clove powder), and highest in control samples at all storage periods. Similar results were reported in ground pork at 5°C by Han *et al.* (2000). The antioxidant effect of the nitrite in cured meat is due to the formation of strong complex with haem Fe, which in turn prevents the release of non-haem Fe and helps in delaying lipid oxidation (Gordon, 1990). Cinnamon, garlic and clove showed significantly higher antioxidative effect than control. The reduction in TBARS values in garlic may be due to allin which is cleaved by allinase enzyme, upon homogenization of garlic to form ammonium pyruvate and 2 propenyl sulfenic acid, the later compound undergoes self-condensation to yield the diallyl thiosulfinate allicin which provides the garlic with its odour or flavour and is often ascribed to its antioxidant activity (Vipraja *et al.*, 2008). The inhibition mechanism of eugenol to lipid peroxidation is due to its interference with the chain reaction by trapping the active oxygen and further it is metabolized to dimmer and the dimeric compound (dieugenol), which inhibits lipid peroxidation at the level of propagation of free radical chain reaction like α -tocopherol (Masahiro *et al.*, 2000). The antioxidant activity of cinnamon may be attributed to the scavenging capacity of cinnamaldehyde which can scavenge 2, 2-diphenyl 1 picryl hydrazyl radical (DPPH) and the hydroxyl radical (OH radical) (Erich, 2006). The results were in agreement with Naveena *et al.* (2006) in buffalo meat, and Singh *et al.* (2014) in raw chicken mince emulsion at refrigerated storage, and Dwivedi *et al.* (2006) in cooked ground beef.

Table 2: Mean (\pm SE, n=6) values of pH and TBA values in garlic, cinnamon, clove and nitrite treated and control minced chicken at different storage periods (4 \pm 1°C)

Biochemical Parameter	Storage days	Control (C)	3%Garlic paste (T1)	2 % w/w Cinnamon powder(T2)	0.2 % w/w Clove powder (T3)	200 ppm Nitrite (N)
pH	0	5.775 \pm 0.02 ^{cs}	5.548 \pm 0.02 ^{es}	5.611 \pm 0.01 ^{ds}	6.175 \pm 0.02 ^{ar}	5.936 \pm 0.05 ^{bs}
	3	5.860 \pm 0.01 ^{cr}	5.750 \pm 0.03 ^{dr}	5.706 \pm 0.02 ^{er}	6.133 \pm 0.06 ^{as}	6.050 \pm 0.03 ^{br}
	6	6.050 \pm 0.03 ^{cq}	5.958 \pm 0.03 ^{dq}	5.905 \pm 0.01 ^{eq}	6.293 \pm 0.02 ^{ap}	6.160 \pm 0.03 ^{bp}
	9	6.213 \pm 0.03 ^{bp}	6.171 \pm 0.01 ^{cp}	6.168 \pm 0.02 ^{dp}	6.270 \pm 0.01 ^{aq}	6.140 \pm 0.03 ^{eq}
TBA values	0	0.436 \pm 0.01 ^{as}	0.337 \pm 0.001 ^{es}	0.388 \pm 0.002 ^{cs}	0.401 \pm 0.001 ^{bs}	0.371 \pm 0.001 ^{ds}
	3	0.937 \pm 0.009 ^{ar}	0.424 \pm 0.002 ^{er}	0.471 \pm 0.002 ^{cr}	0.487 \pm 0.002 ^{br}	0.460 \pm 0.003 ^{dr}
	6	1.102 \pm 0.01 ^{aq}	0.657 \pm 0.002 ^{eq}	0.697 \pm 0.002 ^{cq}	0.701 \pm 0.002 ^{bq}	0.691 \pm 0.003 ^{dq}
	9	2.157 \pm 0.01 ^{ap}	0.848 \pm 0.002 ^{ep}	1.018 \pm 0.01 ^{cp}	1.109 \pm 0.001 ^{bp}	0.958 \pm 0.007 ^{dp}

Mean \pm SE values with different superscripts between rows (p, q, r, s) and between columns (a, b, c, d) for a trait differ significantly ($p < 0.05$).

CONCLUSION

The present study made an attempt to compare the antimicrobial properties of chicken mince treated with spices like Garlic (3 % paste), Cinnamon (2 % w/w powder) and Clove (0.2 % w/w powder with Nitrite (200 ppm) and stored at refrigeration. All three herbal treatment were significantly effective ($p \leq 0.05$) against *Escherichia coli*, *Staphylococcus aureus* and *Clostridium perfringens* with reduced pH and TBA values at all storage intervals from day 0 to 10. Clove, cinnamon powder, and garlic paste had good antimicrobial activity than control against food pathogenic bacteria. Nitrite was most effective against all treated samples followed by clove powder, garlic paste and cinnamon powder in chicken mince for having noble antioxidant and antimicrobial activity. Hence nitrite could be replaced with these herbs for preservation of meat and meat products, but further research work in this direction is required to confirm *in-vivo* and *in-vitro* results in future.

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