Veterinary World, EISSN: 2231-0916
Available at www.veterinaryworld.org/Vol.7/June-2014/14.pdf

RESEARCH ARTICLE
Open Access

Antioxidant and antimicrobial effects of condiments paste used as nitrite replacer in chicken mince

Meena Goswami, P. P. Prabhakaran and Vinay Kumar Tanwar

Department of Livestock Products Technology, College of Veterinary Sciences and Animal Husbandry, Govind Ballabh Pant University of Agriculture and Technology, (GBPUA&T), Pantnagar - 263145, Uttarakhand, India
Corresponding author: Meena Goswami, Present address: Department of Livestock Products Technology, College of Veterinary Sciences and Animal Husbandry, Uttar Pradesh Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan (DUVASU), Mathura-281001, Uttar Pradesh, India; email: dr.goswami2008@yahoo.co.in
PPR: ppkaran_meat@yahoo.co.in, VKT: vktanwarlpt@yahoo.co.in
Received: 10-04-2014, **Revised:** 23-05-2014, **Accepted:** 27-05-2014, **Published online:** 27-06-2014

doi: 10.14202/vetworld.2014.432-438
How to cite this article: Goswami M, Prabhakaran PP and Tanwar VK (2014) Antioxidant and antimicrobial effects of condiments paste used as nitrite replacer in chicken mince, *Veterinary World* 7(6): 432-438.

Abstract

Aim: The present study was conducted to evaluate the antioxidant and antimicrobial effects of 4% Ginger + 4% Garlic + Turmeric 1000ppm (GGT), nitrite 200ppm (N) and the control (C) on minced chicken stored at 4±1^{0}C.

Materials and Methods: Physico-chemical properties (pH, Thio Barbituric acid value (TBA), Peroxide value (PV) and Free Fatty acid (FFA) were evaluated on 0, 3, 6 and 9th day of the storage. The antimicrobial studies *viz.* Total viable count (TVC), *Escherichia coli* count, *Clostridium sporogenes* count and *Clostridium perfringens* count were carried out on 1, 4, 7 and 10th day of storage.

Results: Highly significant difference (P<0.01) was noticed between the treatments and between the storage periods in pH (5.951±0.02-GGT as compared to C-5.898±0.05and N-5.899±0.02), TBA(0.566±0.09 GGT as compared to C-1.569±0.04m and N-0.614±0.11), PV(1.679±0.18-GGT as compared to C-2.595±0.41and N-2.03±0.21), FFA(1.199±0.21-GGT as compared to C-2.284±0.40 and N-1.446±0.24 and N-7.194±0.68), TVC (log CFU/g) (7.528±0.70-GGT as compared to C-8.583±0.49 and N-6.446±0.53), *Escherichia coli* (log CFU/g) (6.476±0.54-GGT as compared to C-7.658±0.71 and N-6.609±0.61), *Clostridium sporogenes* count (log CFU/g) (7.746±0.69-GGT as compared to C-8.681±0.74 and N-) and *Clostridium perfringens* count(log CFU/g) (6.864±0.76-GGT as compared to C-8.790±0.53 and N-6.864±0.58).

Conclusion: The Ginger garlic turmeric paste has an excellent potential to replace the nitrite as a natural antioxidant with other advantages. It is highly effective against *Escherichia coli.* It is also significantly effective against *Clostridium perfringens, Clostridium sporogenes* and TVC as compared to C but lesser than N. We recommend further research by replacing the nitrite with various combinations of the spices and the condiments.

Keywords: *Clostridium perfringens, Clostridium sporogenes, Escherichia coli,* ginger, garlic, turmeric, nitrite.

Introduction

Nitrite is one of the food additives, which is widely used currently in the processed meat manufacturing. Nitrite is one of the important ingredients in curing of the meat products along with salt, sugar, ascorbate and polyphosphate. Nitrite contributes for the cured flavor and cured color due to the formation of nitric oxide myochrome [$(NO)_2$MC] in the cooked meat products and it also inhibits growth of several undesirable bacteria such as *Clostridium sporogenes* [1]. However, the guidelines for the healthy nutrition published by the World Cancer Research Fund suggested to limit the intake of the meat and especially to avoid the processed meat because Nitrite forms N-nitrosamine compounds with the secondary amines in the acidic environment of the stomach, which are known to be potent carcinogens. It also causes conversion of hemoglobin to methmyoglobin which lacks oxygen transporting ability [2].

Several approaches have been tried to replace the nitrite in the meat products. They include the use of betalein, *Staphylococus xylosus* bacteria [3], citrus co products [4] and tea catechins [5] etc. However; nitrite is still widely used in the processed meats because of its potent antibotulinal action which enhances the food safety. Various phytochemicals obtained by the spices offer a promising alternative to the chemical additives for ensuring the meat safety. Inhibitory activity of the spices and their derivatives on the growth of the bacteria, yeasts, fungi and microbial toxin synthesis has been previously reported [6, 7, 8]. Hence, they can be used for meat preservation as main or adjuvant antimicrobial compounds to ensure the production of microbiologically stable foods. Turmeric is one of the spices which is widely used in Indian culinary practices. Curcumin and hydroxyl curcumin are alkaloids present in turmeric which possesses antioxidant, antimicrobial and therapeutic properties [9]. Recently, Sharma *et al* [10] reported that the turmeric when used in raw chicken mince was effective against *Staphylococcus aureus* and exhibited potent antioxidant properties

comparable or superior to the nitrite and ascorbic acid. Huhtanen [11] reported that oleoresin content of the few spices including the turmeric was highly effective against *Clostridium botulinum* in pure culture by disc diffusion method. Garlic is also used as a seasoning agent or condiment in various meat products. Garlic yields allicin on chopping which is a powerful antibiotic and also an anti-fungal compound (phytoncide). Ginger is also an integral part of Indian cooking methods both for vegetarian as well as for non-vegetarian products. Its active ingredients are gingerol and hexahydrocurcumin which prevent the oxidation of oils and fats [12].

Hence, the present investigation was carried out to compare the antioxidative and antimicrobial properties of the ginger garlic turmeric paste with the nitrite in chicken mince.

Materials and Methods

Ethical approval: Permission was obtained from the University Ethics Committee, GABPUA&T, Pantnagar, Uttarakhand.

Birds and materials: Live birds were procured from Instructional Poultry Farm, Nagla, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar and were brought to the Department of Livestock Products Technology. Slaughtering and dressing were carried out following the standard and humane procedures. The carcasses were kept in refrigerator at 4±1^{0}C for 24 hours. The chilled carcasses were deboned on next day and lean meat was then stored at -20^{0}C till further use. Turmeric powder of "Agmark" grade was purchased from the local market for use in the experiments. It contained 2.5% oleoresin content as determined by the ether extraction method. All the chemicals used in the study were of analytical grade and were procured from Hi Media laboratories (P) Ltd, Mumbai. The culture media used in the study were procured from Hi Media Laboratories (P) Ltd, Mumbai and Tulip Diagnostics (P) Ltd, Goa, India. The low density Polyethylene (LDPE) bags were obtained from the local market and sterilized by exposing to UV light for 30 minutes before use.

Preparation of standard bacterial inoculums: The following standard pure cultures *viz. Clostridium sporogenes* (MTCC- 1349), *Clostridium perfringens* (MTCC-450) and *Escherichia coli* (MTCC-1687) were procured from Microbial Type Culture Collection Centre, Institute of Microbial Technology, Chandigarh, India and were used in the study. These cultures were revived under the aseptic conditions as per the instructions.

Preparation and portioning of meat mince: Approximately 1.2 kg of boneless chicken was defrosted before use. The deboned chicken was minced by passing twice through 9mm and 4mm plates of presterilized meat mincer (Sirman® TC-32, Italy). Then minced meat was divided into approximately three equal portions and assigned as the following treatments: control (C), turmeric @1000ppm added along with 4%ginger and 4%garlic (GGT) and sodium nitrite added @ 200ppm (N).

The turmeric (1000ppm to chicken mince) was weighed accurately and dissolved in water (10% by weight of chicken mince) and then incorporated in the chicken mince thoroughly by kneading or massaging for uniform distribution. For the heated turmeric, 1000ppm of turmeric was calculated, accurately weighed and then dissolved in water (10% by weight of chicken mince) as above and then boiled for 15 minutes on a hot plate. The rate of heating was kept uniform between the replications. Each treatment (C, T and GGT) portion was further sub divided into two parts of approximately 300g; one part for studying the physico-chemical properties and the second part for various microbiological studies.

The portion of minced chicken intended for the microbiological studies was further subdivided into four parts, accurately into 100 g portions; for TVC, *Escherichia coli*, *Clostridium perfringens* and *Clostridium sporogenes* count.

Incorporation of bacterial inoculums into the minced chicken: The broth containing known concentrations of *Clostridium sporgenes, Escherichia coli* and *Clostridium perfringens* stored in refrigerator were serially diluted separately with normal saline solution to approximately 107 bacterial colonies per ml. 1ml of each of the inoculums was added to 100 gram of the minced chicken (stored in sterilized LDPE bags) separately and then thoroughly mixed by the kneading so that a final concentration of approximately 105 CFU/gram of the meat mince could be obtained. The concentrations of bacterial cultures were kept deliberately high.

Packaging and storage: The LDPE bags containing inoculated chicken mince were sealed with the help of a sealer (Singhal®, HSP-200, India). The minced portions assigned for the antioxidant and physico-chemical properties, were also sealed in LDPE bags and both were stored at refrigeration temperature (4±1^{0}C) untill further analysis. A total of 6 replications were carried out with each analysis done in duplicate.

The physico-chemical properties were evaluated on 0, 3, 6, and 9th day of storage as per AOAC [13] where as antimicrobial studies were carried out on 1, 4, 7 and 10th day of storage as per APHA [14]. The following abbreviations were used: Control-C; Turmeric 1000ppm + 4% Garlic + 4% Ginger-GGT and 200ppm Nitrite-N.

Statistical analysis: The data were analyzed using ANOVA technique by Snedecor and Cochran [15].

Results and Discussion

The present investigation was carried out to compare various antioxidant and antimicrobial properties of ginger garlic turmeric paste as a nitrite

Table-1: Mean±S.E. values of various physico-chemical properties for different treatments and storage period

Parameters	Treatments	Storage period				
		0 Day	3 Day	6 Day	9 Day	Treatment mean
pH	C	5.755±0.01^{a}	5.838±0.01^{b}	5.922±0.02^{cd}	6.078±0.04^{e}	5.898±0.05^{Y}
	T	5.763±0.01^{a}	5.810±0.02^{ab}	5.872±0.01^{bc}	6.014±0.03^{d}	5.865±0.04^{X}
	GGT	5.888±0.02^{bc}	5.935±0.02^{cd}	5.978±0.01^{d}	6.027±0.02^{de}	5.951±0.02^{Z}
	N	5.832±0.02^{b}	5.878±0.02^{bc}	5.905±0.02^{c}	5.981±0.03^{d}	5.899±0.02^{Y}
	Storage mean	5.825±0.05^{A}	5.883±0.03^{B}	5.935±0.03^{C}	6.028±0.03^{D}	
TBA	C	0.488±0.005^{b}	0.977±0.05^{e}	1.469±0.04^{f}	3.343±0.09^{g}	1.569±0.04^{Z}
	T	0.387±0.008^{ab}	0.514±0.02^{b}	0.710±0.02^{c}	1.006±0.01^{e}	0.654±0.11^{X}
	GGT	0.340±0.005^{a}	0.424±0.01^{ab}	0.653±0.02^{c}	0.848±0.02^{d}	0.566±0.09^{X}
	N	0.364±0.003^{ab}	0.459±0.008^{b}	0.687±0.01^{c}	0.945±0.008^{d}	0.614±0.11^{Y}
	Storage mean	0.397±0.06^{A}	0.620±0.25^{B}	0.936±0.33^{C}	1.712±1.15^{D}	
PV	C	1.366±0.03^{b}	2.450±0.06^{g}	2.900±0.03^{h}	3.666±0.04^{j}	2.595±0.41^{Z}
	T	1.146±0.02^{ab}	1.683±0.09^{cd}	2.016±0.08^{ef}	2.616±0.06^{g}	1.865±0.26^{X}
	GGT	1.100±0.02^{a}	1.650±0.05^{c}	1.816±0.04^{d}	2.150±0.06^{f}	1.679±0.18^{W}
	N	1.305±0.02^{b}	2.011±0.02^{ef}	2.200±0.04^{f}	2.800±0.05^{h}	2.03±0.21^{Y}
	Storage mean	1.257±0.11^{A}	2.037±0.32^{B}	2.305±0.44^{C}	2.872±0.62^{D}	
FFA	C	0.981±0.03^{b}	2.220±0.04^{e}	2.832±0.04^{g}	3.102±0.03^{h}	2.284±0.40^{Z}
	T	0.731±0.01^{a}	1.268±0.05^{c}	1.760±0.01^{d}	2.562±0.04^{f}	1.580±0.33^{X}
	GGT	0.684±0.01^{a}	0.945±0.02^{b}	1.335±0.05^{c}	1.834±0.09^{d}	1.199±0.21^{Y}
	N	0.765±0.02^{a}	1.204±0.03^{c}	1.770±0.07^{d}	2.044±0.02^{e}	1.446±0.24^{W}
	Storage mean	0.810±12^{A}	1.456±0.55^{B}	1.979±0.62^{C}	2.326±0.55^{D}	

Different superscripts between columns (A, B, C, D….) and between rows (W, X, Y, Z……) show significant (P<0.05) different in between storage and treatments respectively; Different superscripts (a, b, c, d…..) differ significantly (P<0.05) show interaction between different treatments and storage

replacer in chicken mince stored at 4±1^{0}C upto 10 days of storage.

pH: The analysis of variance indicated a highly significant difference (P<0.01) in pH between treatments and between storage periods (Table-1). However, the interaction between treatments and storage periods showed no significant difference (P>0.05). Addition of N did not have significant effect (P>0.05) on pH of chicken mince, where as GGT treated minced meat had significantly higher (P<0.01) mean pH values as compared to C and N. Mathew *et al* [16] found no significant difference in pH between control and 200ppm nitrite added buffalo meat chunks stored at 4±1^{0}C for 20 days.

Reddy *et al* [17] also found significant increase (P<0.01) in pH of chicken meat balls with 6% ginger during storage at -20±2^{0}C for 60 days. Krishnan *et al* [18] observed higher pH and enhanced shelf life in raw chicken meat with incorporation of spice mix under refrigeration storage. Ginger extract (2%) treated microwave oven cooked chevon patties had significantly higher pH than control samples [19]. Therefore, it is highly possible that the ginger and garlic present in GGT treated mince samples might have increased the pH and not the T. Yumin *et al* [20] found increased pH values of stewed pork prepared with incorporation of ginger, onion and garlic under refrigerated storage. Overall, storage mean pH of chicken mince increased significantly (P<0.01) with the progression of the storage period. Kumar and Sharma [21] attributed the increase in pH during storage of low fat pork patties to proteolysis due to bacterial growth. Vijaykumar and Biswas [22] reported a significant increase in pH of enrobed duck cutlets stored at refrigeration temperature for 21 days.

Thio Barbituric acid (TBA) value (mg malonaldehyde/kg): The analysis of variance indicated highly significant difference (P<0.01) between treatments, between storage periods as well as for the interaction between treatments and storage period (Table-1). The overall mean TBA value for GGT and N were significantly lower (P<0.01) as compared to C.

Han *et al* [23] recorded that 100ppm nitrite treated ground pork samples stored at 5^{0}C showed higher antioxidative effect than control. 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 of lipid oxidation [24]. Curcuminoids in turmeric scavenge free radicals at the cost of becoming free radicals themselves. These second hand free radicals are unreactive products and are short lived, so the lipid oxidation reaction is terminated. Reema *et al* [19] evaluated the antioxidant properties of ginger and found that 2% ginger extract treated microwave oven cooked chevon patties had significantly lower TBA values than control samples when stored at 0-2^{0}C for 28 days. Oyas *et al* [25] also reported that ethyl acetate extract of spices like ginger, garlic, turmeric and cinnamon have got profound antibacterial and antioxidant effect in fish meat preservation. The overall mean TBA values were significantly (P<0.01)

Table-2: Mean±S.E. values of various microbiological properties for different treatments and storage period

Parameters	Treatment	1 Day	4 Day	7 Day	10 Day	Treatment mean
Total plate count	C	6.282±0.04^{c}	8.335±0.01^{g}	9.435±0.02^{ij}	10.279±0.16^{k}	8.583±0.49^{Z}
	T	5.867±0.09^{b}	7.334±0.07^{d}	8.673±0.12^{h}	9.634±0.04^{j}	7.877±0.70^{Y}
	GGT	5.397±0.09^{a}	7.287±0.05^{e}	8.177±0.19^{g}	9.249±0.05^{i}	7.528±0.70^{X}
	N	5.163±0.02^{c}	6.934±0.23^{d}	7.789±0.13^{f}	8.892±0.11^{h}	7.194±0.68^{W}
	Storage mean	5.614±0.48^{A}	7.518±0.59^{B}	8.467±0.70^{C}	9.473±0.58^{D}	
Escherichia coli	C	5.949±0.11^{b}	6.694±0.05^{d}	8.375±0.08^{g}	9.615±0.05^{i}	7.658±0.71^{Z}
count	T	5.045±0.04^{a}	6.300±0.07^{c}	7.106±0.07^{e}	8.667±0.11^{h}	6.780±0.65^{Y}
	GGT	5.033±0.02^{c}	5.914±0.09^{b}	6.880±0.07^{de}	7.994±0.09^{d}	6.476±0.54^{X}
	N	5.129±0.03^{c}	5.877±0.05^{b}	6.803±0.07^{d}	7.974±0.09^{f}	6.446±0.53^{X}
	Storage mean	5.370±0.41^{A}	6.161±0.39^{B}	7.352±0.72^{C}	8.527±0.76^{D}	
Clostridium	C	6.386±0.04^{d}	8.434±0.01^{g}	9.545±0.05^{i}	10.357±0.06^{j}	8.681±0.74^{Z}
sporogenes count	T	5.789±0.08^{b}	7.325±0.07^{f}	8.372±0.01^{g}	9.277±0.06^{h}	7.691±0.65^{Y}
	GGT	5.656±0.10^{b}	7.433±0.07^{f}	8.479±0.05^{g}	9.417±0.03^{hi}	7.746±0.69^{Y}
	N	4.956±0.09^{a}	6.111±0.04^{c}	7.041±0.08^{e}	8.326±0.06^{g}	6.609±0.61^{X}
	Storage mean	5.666±0.58^{A}	7.326±0.95^{B}	8.355±1.02^{C}	9.366±0.82^{D}	
Clostridium	C	6.337±0.04^{b}	8.457±0.04^{d}	9.917±0.10^{h}	10.409±0.02^{i}	8.790±0.53^{Z}
perfringenes count	T	5.335±0.03^{c}	8.284±0.05^{d}	9.302±0.05^{f}	9.662±0.05^{g}	8.146±0.84^{Y}
	GGT	5.397±0.02^{c}	7.551±0.02^{c}	8.879±0.17^{e}	9.332±0.03^{f}	6.864±0.76^{X}
	N	5.203±0.007^{c}	6.384±0.02^{b}	7.574±0.17^{c}	8.293±0.03^{d}	6.864±0.58^{X}
	Storage mean	5.645±0.49^{A}	7.464±0.84^{B}	8.790±0.95^{C}	9.344±0.86^{D}	

Different superscripts between columns (A, B, C, D….) and between rows (W, X, Y, Z……) show significant (P<0.05) different in between storage and treatments respectively; Different superscripts (a, b, c, d…..) differ significantly (P<0.05) show interaction between different treatments and storage

increased during the refrigeration storage in all the treatments. Nayak and Tanwar [26] attributed the increase in the TBA values with the advancement of storage period which was due to increased lipid oxidation and the production of volatile metabolites in the presence of oxygen.

Peroxide value (PV): The overall mean PV for N and GGT were significantly lower (P<0.01) than C. Highly significant difference (P<0.01) was found in between GGT and N treated chicken mince (Table-1). It may be because of composition of the nitrite where one part of nitrogen is present in combination with two parts of oxygen. Chan *et al* [27] found that turmeric extract was an effective antioxidant in chicken mince stored at refrigerated temperature. Sreejayan *et al* [9] reported that curcumin is a potent inhibitor of lipid peroxidation catalyzed by Fe and its chelates in rat brain homogenate and rat liver microsomes. Dzudie *et al* [28] evaluated the antioxidant properties of ginger and found that the beef patties containing 0.2% level of ginger essential oils showed the best characteristics in relation to lipid oxidation as compared to ground oil and animal fat.

Free fatty acid (FFA) value: The analysis of variance indicated highly significant difference in FFA value between treatments, between storage periods and for the interaction between treatments and storage periods (Table-1).The overall mean FFA value for N and GGT were significantly lower than C. GGT had significantly lower overall mean FFA value than N. Mathew *et al* [16] observed that nitrite treated buffalo meat chunks had significantly lower FFA values than control and were acceptable up to 20 days when stored at refrigeration temperature.

Chan *et al* [27] observed that turmeric had significantly higher antioxidant effect in minced poultry meat as compared to control during storage at 3^{0}C for 9 days. El-Ghorab *et al* [29] studied the antioxidant properties of cumin ginger and garlic and stated that exogenous addition of these natural spices in food reduced the lipid oxidation by reducing Fe+3 and free radicals scavenging activities. Isothiocynates present in ginger and garlic inactivated and modified several enzymes in meat such as 3-hydroxy-3-methyl glutaryl CoA reductase, glutathione-S-transferase and catalase, resulting in the prevention of lipid oxidation. Overall, storage mean FFA values were increased significantly during the whole storage period in all treatments. Kanatt *et al* [30] reported that lipolysis of meat during storage by the action of tissue enzymes and lipolytic enzymes, obtained from spoilage micro organisms led to the formation of FFA.

Total viable count (TVC) (log CFU/g): The overall mean TVC (log CFU/g) for N and GGT were significantly lower when compared to C. However, highly significant difference was observed between GGT and N (Table-2). The total viable count was found exceptionally high in C on 1st day due to less satisfactorily dressing and deboning procedure followed in the experiment. Ockerman [31] stated that Sodium nitrite inhibited growth of bacteria and delayed the microbial spoilage of cured meat products.

Khanna [6] reported that Curcumin and other curcuminoids inhibit the growth of various bacteria like *Staphylococcus aureus, Salmonella paratyphi,*

Trichphyton gypseum and *Mycobacterium tuberculosis* in concentration varying from 1 in 20000 to 1 in 640000. Neogi *et al* [32] studied synergistic combination of various spices and reported that garlic and turmeric (70% ethanolic extract) were highly effective against *Staphylococcus aureus* and *Salmonella typhi.* Sagufta *et al* [33] evaluated antimicrobial properties of different varieties of turmeric against *Bacillus subtilis, Bacillus macerans, Bacillus licheniformis* and *Azotobacter* using agar well diffusion method and observed that the MIC values for different strains and varieties ranged from 3.0 to 20.6 mm in diameter.

Sunilson *et al* [34] evaluated the antimicrobial properties of *Curcuma longa* and *Zingiber officinale* and found that methanol extracts (100µg/ml) of these two had highly significant reduction in common food borne bacteria like *Escherichia coli, Salmonella entriditis, Clostridium prerfringnes, Staphylococcus aureus, Campylobactor jejuni* and *Bacillus cereus* etc. Isothiocynates present in ginger and garlic can inactivate the extracellular enzymes through the oxidative cleavage of disulphide bonds necessary for survival of various bacteria [24]. Overall, storage mean for TVC increased significantly on storage period for all treatments.

***Escherichia coli* count:** The analysis of variance indicated highly significant difference in *Escherichia coli* count between treatments, between storage periods as well as for the interaction between treatments and storage periods (Table-2). Overall mean *Escherichia coli* count (log CFU/g) were significantly lower for N and GGT when compared to C. However no significant difference was found between GGT and N. Nakajima *et al* [35] reported a significant reduction in *Escherichia coli* count when added with nitrite as compared to control in chicken meat. Morita and Hiroshi [36] explained that the antibacterial activity of NO derived from sodium nitrite against *Escherichia coli* 0157:H7 was due to the 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 in the cells. Neogi *et al* [8] reported that combination of garlic and turmeric (70% ethanolic extract) was synergistic and much effective against *Escherichia coli.* Sana and Ifra [37] evaluated antibacterial activity of aqueous and ethanol extracts of spices and reported that among the selected spices garlic had best inhibitory activity with a zone of 22mm against *Escherichia coli*. Tajkarimi *et al* [38] observed anti microbial activity of garlic and turmeric against *Escherichia coli* and averted herbs and spices have strong antimicrobial activity in various complex food systems.

***Clostridium sporogenes* count:** The treatment mean of *Clostridium sporogenes* count for GGT and N were significantly lower when compared to C. N was found to be more effective than GGT (Table-2). The effectiveness of nitrite against *Clostridium botulinum* was shown by Jaffery and Andrew [39] who stated that antibotulinal effect in thermally processed meat products takes place at two different stages in the life cycle of the microorganism. The first *Clostridium botulinum* controlling effect of nitrite is the inhibition of vegetative cells emerging from surviving spores. The second controlling effect is preventing cell division in any vegetative cells. In present study, GGT was found to be significantly more effective than C. Madhumita *et al* [40] found that paste of garlic turmeric and cinnamon with gum Arabic as binding agent enhanced the shelf life of minced meat when used as bio edible coating. Haiying *et al* [41] reported antimicrobial activity of various spices including turmeric rhizome, garlic and ginger rhizome against *Clostridium sporogenes* as a nitrite replacer. Storage mean of *Clostridium sporogenes* count increased significantly on all storage days. In the present investigation, *Clostridium sporogenes* was selected to study because it shares almost all the physiological properties with *Clostridium botulinum* and produces more heat resistant spores than the later.

***Clostridium perfringens* count:** The analysis of variance indicated highly significant difference in *Clostridium perfringens* count between treatments, between storage periods and for the interaction between treatments and storage periods (Table-2). Treatment mean of *Clostridium perfringens* count for GGT and N were significantly lower as compared to C. However, no significant difference was found between N and GGT. Mauricio *et al* [42] showed antimicrobial activity against *Clostridium perfringens* on addition of sodium nitrite and erythrobate in ham. Storage mean of *Clostridium perfringens* count increased significantly on all storage days.

Zarringhalami *et al* [43] reported that nitrite could be partially replaced with annatto with better color values but antimicrobial properties of nitrite were better than annatto against *Clostridium perfringens*. Goswami *et al* [44] observed that ginger garlic and turmeric paste and heat treated turmeric had comparable activity with nitrite against *Clostridium perfringens.* Hoi [45] reported that methanol extract of turmeric rhizomes had strong antibacterial activity against *Clostridium perfringens* at a concentration of 5.0 mg/disc than control by disc diffusion method. Sunilson *et al* [33] evaluated the invitro antibacterial activity of *Curcuma longa* and *Gingiber officinale* and observed that methanol extracts (100µg/ml) of these spices had highly significant reduction against *Clostridium perfringens.*

Conclusion

We conclude from the present study that the ginger garlic and turmeric paste had better antioxidant properties than the nitrite in a complex food system like chicken mince. It can replace the nitrite as a strong natural antioxidant. It had very good antimicrobial

activity against the total viable count but the effect was lower than the nitrite. GGT had an antimicrobial effect equal to the nitrite against *Escherichia coli*. GGT had a better antimicrobial effect against *Clostridium sporogenes* and the effect was slightly lower than the nitrite. However, the antimicrobial effect against *Clostridium perfringens* was the same for both the nitrite and the GGT. These findings revealed that there is a great possibility for the replacement of the nitrite with the natural spices and the condiments like ginger, garlic, turmeric etc. However, we recommend further research work in this direction using new variations by combinations of various spices and condiments with well established antioxidant and antimicrobial properties.

Authors' contributions

The present research work is the part of MG's thesis work. MG collected literature and prepared the initial version of manuscript. PPP and VKT helped for carried out research and revised manuscript for critical scientific corrections. All authors read and approved the final manuscript.

Acknowledgements

The authors are thankful to College of Veterinary Sciences and Animal Science, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India for providing necessary fund and facilities for this study.

Competing interests

The authors declare that they have no competing interests.

References

1. Gulcemal, Y., Nevin, O., Ayca, O. and Filiz, S. (2014) Voltametric determination of nitrite in meat products using polyvinylimidozole modified carbon paste electrode. *Food Chem,* 152: 245-250.
2. Tonu P. (2013) Toxicolgical issues associated with production and processing of meat. *Meat Sci,* 95:844-853.
3. Peijun, L., Baohua, K., Qian, C., Dongmei, Z. and Ning, L. (2013) Formation and identification of nitrosomyoglobin by *Staphylococus xylosus* in raw meat batters: a potential solution for nitrite substitution in meat products. *Meat Sci,* 91(1): 67-72.
4. Viuda-Martos, M., Fernandez, J. L., Sayas, E. B., Sendra, E., Navarro, C. and Perez-Alvarez, J. A. (2009) Citrus coproducts as technological strategy to reduce residual nitrite content in meat products. *J. Food Sci,* 74 (8): 93-100.
5. Moawad, R. K., Wafaa, A. and Nadir, A. S. (2012) Effect of nitrite level and tea catechins on residual nitrite and quality indices of raw cured sausages. *J. Appl. Sci Res*, 8(2): 815-822.
6. Khanna. N. M. (1999) Turmeric- nature's precious gift. *Curr. Sci*, 76(10): 1351-1356.
7. Negi, P. S., Jayarakasha, G. K., Jagan Mohan, R. L. and Sakariah, K. K. (1999) Antibacterial activity of Turmeric oil: A byproduct from Curcumin manufacture. *J. Agric. Food Chem*, 47(10): 4297-4300.
8. Sethi, V and Meena. M. R. (1994) Antimicrobial activity of essential oils from spices. *J. Food Sci. Tech,* 31(1): 68-70.
9. Sreejeyan, M. N. and Rao, M. N. A. (1994) Curcuminoids as potent inhibitors of lipid peroxidation. *J. Pharma. Pharmacol*, 46(12): 1013-1-16.
10. Sharma, J., Prabhakaran, P., Tanwar, V.K., Das, S. K. and Goswami, M. (2012) Antioxidant effect of turmeric powder, nitrite and ascorbic acid on stored chicken mince. *Int. J. Food Sci. Technol,* 47(1):61-66.
11. Huhtanen, C.N. (1980) Inhibition of *Clostridium botulinum* by spices extract and aliphatic alcohols. *J. Food Protect*, 43(3): 195-196.
12. Tiwari V., Shankar R., Srivastava J and Padama S. V. (2006) Change in antioxidant activity of spices- turmeric and ginger on heat treatment. *Elec. J. Env. Agricult. Food Chem,* 5(2): 1313-1317.
13. AOAC (1992) Official methods of analysis 14th edition, Association of official analytical chemists. Washington D.C. p367-384.
14. APHA (1984) Compendium of methods for the microbiological examination of foods. 4th edition C. V andergrant and D. F. S plittstoesser. American Public Health Association, Washington, D.C p 919-927.
15. Snedecor, G.W. and Cochran, G.W. (1989) Statistical methods, 8th edition. Iowa State University Press, Ames, Iowa. p88-92.
16. Mathew, T., Anjaneyulu, A. S. R., Thomas, R. and Kondaiah, N. (2008) Effect of nitrite levels on the quality of smoked buffalo meat chunks and their shelf life during refrigerated storage. *J. Food Sci. Tech,* 45 (4): 317-322.
17. Naveena, B. M. and Mendiratta, S. K. (2001) Ginger as a tenderizing, antioxidant and antimicrobial agent for meat and meat products. *Indian Food Industry*. 20 (6): 47-49.
18. Krishnan, K. R., Babuskin, S., Babu, P. A. S., Sasikala, M., Sabina, M., Archana, G., Sivrajan, M and Sukumar, M. (2014) Antimicorbial and antioxidant effects of spice extracts on the shelf extension of raw chicken meat. *Int. J. Food Microbiol,* 171: 32-40.
19. Reema, R., Sahoo, J., Hooda, S. ANd Karwasra, R. K. (2005) Effect of ginger extract and cooking methods on oxidative stability and physicochemical quality of vacuum packaged cooked chevon patties during storage. *J. Food Sci. Tech,* 42 (3): 275-279.
20. Yumin, C., Weigang, G., Jinjie, Z., Yin, C., Xingquin, Y., Yaqin, H. and Jianchu, C. (2010) Effects of chitosan, aqueous extract of ginger, onion and garlic on quality and shelf life of stewed-prok during refrigerated storage. *Food Chem,* 141 (3):1655-1660.
21. Kumar, M. *and Sharma, B. D.* (2004) The stprage stability and textural physico-chemical and sensory quality of low fat ground pork patties with carrageenan as fat replacer. *Int. J. Food Sci. Technol,* 39: 31-42.
22. Vijaykumar, K. S. and Biswas, S. (2006) Quality and storage stability of enrobed duck cutlet. *J. Food Sci. Tech,* 43 (2): 143-153.
23. Han, S. K., Yamauchi, K. and Park, h. k. (2000) Effect of nitrite on lipid component and lipid oxidation in stored cured meat under refrigeration. *J. Anim. Sci. Technol.,* 42(3): 339-348.
24. Gordon, M. H. (1996) The mechanism of antioxidant action in vitro. *In Food Antioxidant,* Ed by Hudson BJF, Elsevier Applied Science, New York, p1-18.
25. Oyas, A. A., Sahu, N. P. and Pal, A. K. (2013) Antioxidant activity and antimicrobial property of some Indian spices. *Int. J. Sci. Res. Pub.,* 3(3): 1-7.
26. Nayak, N. K. and Tanwar, V. K. (2005) Sensory attributes of tofu incorporated chicken patties. *Beverage and Food World, (January)* 76-78.
27. Chan, S. W., Alina, A. R., Babji, A. S., Chia, K. L. and Nulkirah, M. (2002) Turmeric (*Curcuma domestica*) as an antioxidant agent in minced poultry meat. Towards modernization of research and technology in herbal industries. Proceedings of the Seminar on Medical and Aromatic plants, 222-226.
28. Dzudie. T., Kouebou, C. P., Essia N. J. J. and Mbofung, C. M. F. (2004) Lipid sources and essential oils effect on quality and stability of beef patties. *J. Food Eng,* 65(1):67-72.

29. El-Gharob, H., Nauman, M., Anjum, F. M., Hussain, S. and Nadeem, M. (2010) Comparative study on chemical composition and antioxidant activity of ginger and cumin. *J. Agric. Food Chem.* 58(4): 8231-8237.
30. Kanatt, S. R., Paul, P., D'souza, S. F. and Thomas P. (1998) Lipid peroxidation in chicken meat during chilled storage as affected by antioxidant combined with low-dose Gamma irradiation. *J. Food Sci,* 63(2): 199- 200.
31. Ockerman, H. W. (1990) Chemistry of meat tissue. Thesis submitted to Department of Animal Science, The Ohio state University, Columbus, Ohio.
32. Neogi, U., Ruchi, S. and Bushra, I. (2007) Invitro combinational effect of bio-active plant extracts on common food borne pathogens. *Int. J. Micrbiol. Res,* 2 (5): 500- 503.
33. Shagufta, N., Safia, J., Saiqa, I., Farkhanda, M., Farah, A. and Aamir, A. (2010) Anitbacterial activity of Curcuma longa varities against different strains of bacteria. *Pakistan J. Bot,* 42(1): 455-462.
34. Sunilson, J. A. J., Suraj, R., Rejitha, G., Anandarajagopal, K., Kumari, A. V. A. G. and Promwichit, P. (2009) In vitro antimicrobial evaluation of *Zingiber officinale, Curcuma longa* and *Alpinia galangal* extracts as natural food preservatives. *Am. J. Food Technol,* 4 (5):192-200.
35. Nakajima, H., Sameshima, T., Yamanaka, H., Takeshita, K., Akiyama, S. And Suzuki, A. (1989) Antimicrobial effect of sodium nitrite and sodium chloride in nutrient broth or ground pork meat. *J. Food Hygienic Society Japan*, 30(5): 411-416.
36. Morita, H. and Hiroshi, Y. (2004) Antimicrobial action against verotoxigenic Escherichia coli 0157:H7 of nitrite oxide derived from sodium nitrite. *Biosci. Biotechol. Biochem,* 68 (5):1027-1034.
37. Sana, M. and Ifra, G. (2012) Antibacterial activity of aqueous and ethanolic extracts of garlic, cinnamon and turmeric against *Escherichia coli* ATCC25922 and *Bacillus subtilis* DSM 3256. *Int. J. App. Bio. Pharma. Technol,*3(2): 131-136.
38. Tajkarimi, M. M., Ibrahim, S. A. and Cliver, D. O. (2010) Antimicrobial herbs and spice compounds in food. *Food Control,* 21: 1199-1218.
39. Jeffery, J. S. and Andrew, L. M. (2012) Human safety controversies surrounding nitrate and nitrite in the diet. *Nitric Oxide*, 26:259-266.
40. Madhumita, R., Ramalingum, C., Divyakriti, Y and Bedi, J. (2012) Bioedible coating of emat using garlic, cinnamon and turmeric. *Euro. J. Exper. Bio*, 2(5): 1439-1443.
41. Haiying, C., Alonzo, A. G. and Hiroyuki, N. (2010) Antimicrobial eficacias of plant extracts and sodium nitrite against *Clostridium botulinum. Food Control,* 21:1030-1036.
42. Mauricio, R. S., Carol, V. M., David, A. C., Daniel, D.S., Vijay, K.,J., Dennis, E. B. and Harshvardhan, T. (2013) Effect of meat ingredients (sodium nitrite and erythrobate) and processing (vacuum storage and packaging atmonsphere) on germination and outgrowth of *Clostridium perfringnes* spores in ham during abusive cooling. *Food Microbiol,* 35:108-115.
43. Taormina, P. J. Bartholomew, G. W. and Dorsa, W. J. (2003) Incidence of Clostridium perfringnes in commercially produced cured raw meat product mixtures and behaviour in cooked products during chilling and refrigerated storages. *J. Food Protect,* 66 (1): 72-81.
44. Goswami, M., Prabhakaran, P and Tanwar, V K. (2013) Comparative study of spices and condiments mix as nitrite replacer for reducing microbial load in chicken mince. *J. Meat Sci. Technol,* 1(3): 91-97.
45. Hoi-Seon, L. (2006) Antimicrobial properties of turmeric *(Curcuma longa)* rhizome derived ar-turmerone and curcumin. *Food Sci. Biotechnol,* 15 (4): 559-563.

****