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Antimicrobial Efficacy of Garlic on Food Borne Pathogens in Broiler Meat

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ABSTRACT

Spices have been proven traditionally to be used for food preservation. The present study was conducted to evaluate the efficiency of aqueous extract and essential oil of garlic as decontaminating agents in chicken carcasses. The antimicrobial effect of garlic on *Salmonella typhimurium, E. coli* and *Staphylococcus aureus* was evaluated by challenging the broiler carcasses with specific cultures of test organisms and marinating them with different levels of garlic extract there after. Microbial analysis was carried out after 0, 3, 6, 12, 24 and 48 hours of refrigerated storage (8± 2 ^{0}C). Dipping with aqueous extract of garlic (100%) was found to be more efficient in reducing bacterial pathogens among various treatments. Greater reduction in Total Viable Count (TVC), *Salmonella typhimurium, E. coli* and *Staphylococcus aureus* count was observed in spiked chicken legs inoculated with test organisms and marinated with 2.5 per cent garlic extract.

Key words: *Garlic, antimicrobial effect, chicken meat, food borne pathogen.*

INTRODUCTION

Food safety is an increasingly important public health issue in spite of modern improvements in meat hygiene and production techniques (WHO, 2002a). Hence, there is a need for new methods of reducing or eliminating food borne pathogens. One such possibility is the use of biopreservatives such as spices, their Aqueous Extracts (AE) and Essential Oils (EO) of spices. Spices have traditionally been found to be useful for food preservation as well as for medicinal purposes (Burt, 2004). Many of the commonly consumed spices in the world such as garlic, ginger, cinnamon have been reported to have antibacterial activity due to the active ingredients such as allicin, Sesquiterpenes, Di-allyl-di-sulphide etc. Garlic has a long held reputation as a medicine and has also been used in various food preparations through the ages. The antibacterial properties of crushed garlic have been known for a long time. The antimicrobial effect of garlic is due to allicin. The intact garlic bulb contains the precursor of allicin i.e. alliin (5-allyl-L cysteine-S-oxide). This hydrolyses to allicin, pyruvate and ammonia by phosphopyridoxal enzyme allinase action, on disruption of garlic bulb (Cavallito and Bailey, 1944). Antimicrobial activity of garlic has been reported against several food borne pathogens like *Staphylococcus aureus* (Kyung *et al*., 2002; Lee *et al*., 2003; El-Astal, 2004; Benkeblia *et al*., 2005), *Bacillus cereus* (Saleem and Al-Delaimy, 1982), *Escherichia coli* (Yoon- Soon-Kim *et al*., 1996; Ceylan *et al*., 1998; Sasaki *et al*., 1999), *Salmonella* (Leuschner and Zamparini, 2002; Chung *et al*., 2003; Sharma, 2004; Benkeblia *et al*., 2005), *Vibrio parahaemolyticus* (Chung *et al*., 2003) and *Listeria monocytogenes* (Bank *et al*., 1990; Menon *et al*., 2000; Leuschner and Ielsch, 2003). The isothiocyanates in garlic EOs inactivated extra cellular enzymes through the oxidative cleavage of disulphide bonds (Lambert *et al*., 2001).

So the present study was envisaged to evaluate the antimicrobial activity of garlic on broiler carcass.

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MATERIALS AND METHODS

Preparation of extracts of garlic: Aqueous extract (AE) of garlic was prepared as per the method outlined by Indu *et al.,* (2006).The fresh garlic was obtained from the local market and cleaned. About 100g of garlic was powdered in sterile blender with 100 ml of sterile distilled water. The extract was then sieved through a fine sterile muslin cloth and sterilized using a membrane filter (0.45-micron sterile filter). This sterile aqueous extract obtained was considered as the 100 percent concentration of the extract. The essential oil (EO) of garlic was obtained from M/S Plants lipids Ltd, Cochin, Kerala and it was diluted using ethanol to get 1:150, 1:250 and 1:500 dilutions.

Processing and dilution of samples: All swab samples collected from each area of broiler carcass and surface (4 cm X 4 cm) were put into the tube containing 25 ml of sterile diluent and agitated for five minutes. Similarly, 11 grams of meat from sample was triturated in a sterilized mortar and pestle and transferred to 99 ml of sterile diluent, separately.

Bacterial count: Total viable count (TVC) and count of different bacterial pathogens in meat was enumerated as per the method given by APHA (1992). The reference strains of *Escherichia coli* (MTCC 452), *Staphylococcus aureus* (MTCC3103), *Salmonella typhimurium (*MTCC 1251), *Escherichia coli* O157 (MTCC 452), *Bacillus cereus* (MTCC), *Bacillus subtilis* (MTCC), *Klebsiella pneumoniae* (MTCC) were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh. The cultures were maintained at 4^{o}C in brain heart infusion broth and were tested for purity, morphology and biochemical characteristics every 15 days.

Evaluation of garlic as decontamination agent in chicken carcass: To evaluate the efficiency as decontaminating agent in chicken meat, two concentrations of aqueous extract (100 % and 75 %) and three concentration of essential oil (1:150, 1:250 and 1:500) of garlic were used. Chicken whole leg and breast samples were procured from the market for each treatment. Initial microbial counts of the samples were assessed by serial dilution followed by plating in selective media. The same samples were then dipped into different concentrations of aqueous and essential oils of garlic and were allowed a contact time of two minutes (contact time was standardized based on the efficiency of the extracts to reduce bacterial counts and sensory evaluation). Then samples were drawn from each treatment and microbial counts were evaluated and expressed as $\log_{10}$ cfu/g of meat sample. The difference in log values before and after treatment was used as a guide to assess the antimicrobial and decontaminating ability of spice extracts.

Challenging with Escherichia coli, Staphylococcus aureus and Salmonella typhimurium: Stock cultures of *Escherichia coli, Staphylococcus aureus* and *Salmonella typhimurium* strain were sub cultured once in every 15 days and were prepared on to Mc Conkey agar, Baird Parker agar and Hektoen Enteric agar (HIMEDIA) respectively. Incubation was done at 37^{o}C for 24 hours. Young nutrient broth cultures of 18 hrs, of *Escherichia coli, Staphylococcus aureus* and *Salmonella typhimurium*, were obtained. The chicken whole legs were inoculated with 10^{7} cells/ ml (assessed by Direct microscopic Count) of *Escherichia coli, Staphylococcus aureus* and *Salmonella typhimurium.*

Marination of whole chicken legs with garlic extract: Whole chicken legs inoculated with standard cultures of *E. coli, Staphylococcus aureus* and *Salmonella typhimurium* were marinated with different levels of garlic extract (v/w) and wrapped in aluminum foil and held under refrigerated condition and the microbial analysis was carried out at 0,3,6 12, 24 and 48 hours.

Statistical Analysis: All the experiments were triplicated and the data obtained in the study were analyzed statistically for significance as per the procedure outlined by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Evaluation of AEs and EOs of Garlic as decontamination agent: The log reduction of TVC, *E.coli, Staphylococcus* and *Salmonella* counts at

Table 1: Effect of Aqueous Extract (100 and 75 per cent) and Essential Oil (1:150, 1:250 and 1:500) of Garlic on decontamination in Chicken meat (Mean ± SE) (log10 cfu/g)

		100 %	75 %	1:150	1:250	1:500
TVC	BT	5.77 ± 0.07	5.64 ± 0.07	5.90 ± 0.03	5.73 ± 0.05	5.81 ± 0.02
	AT	4.66 ± 0.02	4.76 ± 0.09	5.26 ± 0.02	5.23 ± 0.05	5.37 ± 0.06
	LR	**1.11 ± 0.06^{a}**	**0.88 ± 0.06^{b}**	**0.63 ± 0.03^{c}**	**0.50 ± 0.02^{d}**	**0.44 ± 0.02^{d}**
E coli	BT	3.42 ± 0.07	3.47 ± 0.08	3.42 ± 0.12	3.35 ± 0.11	3.49 ± 0.03
	AT	2.71 ± 0.05	2.96 ± 0.11	2.77 ± 0.04	3.02 ± 0.15	3.35 ± 0.02
	LR	**0.72 ± 0.03^{a}**	**0.51 ± 0.07^{a}**	**0.52 ± 0.05^{a}**	**0.33 ± 0.06^{ab}**	**0.24 ± 0.05^{c}**
Staphylococcus	BT	4.58 ± 0.06	4.56 ± 0.07	4.72 ± 0.07	4.71 ± 0.06	4.61 ± 0.10
	AT	3.76 ± 0.04	4.05 ± 0.067	4.27 ± 0.07	4.30 ± 0.03	4.38 ± 0.09
	LR	**0.82 ± 0.03^{a}**	**0.51 ± 0.08^{b}**	**0.46 ± 0.04^{bcd}**	**0.41 ± 0.09**	**0.23^{ce} ± 0.02**
Salmonella	BT	3.23 ± 0.04	3.26 ± 0.12	3.34 ± 0.05	3.36 ± 0.18	3.22 ± 0.18
	AT	2.61 ± 0.04	2.76 ± 0.09	2.95 ± 0.08	3.12 ± 0.16	3.09 ± 0.17
	LR	**0.64 ± 0.05^{a}**	**0.50 ± 0.04^{a}**	**0.40 ± 0.08^{b}**	**0.24 ± 0.02^{bc}**	**0.14± 0.01^{d}**

BT: Before Treatment AT: After Treatment LR : Log Reduction
Means bearing different superscripts (a, b, c) within rows differ significantly (P< 0.01)

100 percent and 75 per cent AE concentrations is presented in Table 1. The AE of garlic at 100 percent concentration significantly (P<0.001) reduced TVC and *Staphylococcus* counts in comparison with 75 percent AE, EO 1:150, EO 1:250 and EO 1:500 in decreasing order. But there was no significant difference between 75 and 100 percent AE, 1:150 and 1:250 dilutions of EO of garlic in reducing *E. coli* count where as 75 and 100 percent AE of garlic significantly reduced *Salmonella* count in comparison with EO of garlic at all dilutions. The AE of Garlic was better with respect to brining down the microbial load compared to EO. This might be attributed to fact that allicin, the active principle in garlic is water soluble. Hence, it is well extracted in presence of water. (Ellmorg and Feldberg, 1994). However, Chung *et al* (2003) observed that garlic could only reduce the count of *Staphylococcus aureus*, but could not inactivate in food. Yadav *et al.,* (2002) reported a lower coliform counts in garlic extract treated sample in compared to control.

Effect of marination with garlic extracts of chicken legs spiked with reference strains under refrigeration: Garlic paste at both 1.5 and 2.5 percent concentration significantly (P<0.001) reduced all the microbial counts in comparison with control from 3 hr of incubation itself (Table.2). Marinating spiked chicken legs with 2.5 percent garlic extract significantly reduced *E.coli* counts by 3 hr of incubation, *Salmonella typhimurium* and *Staphylococcus aureus* count by 6hr of incubation and TVC count by 12 hr of incubation in comparison with 1.5 percent garlic extract. But this effect of 2.5 percent garlic extract was absent after 12 hr incubation for *E.coli* and *Salmonella typhimurium* and 24 hr incubation for *Staphylococcus aureus* and TVC and after that the antimicrobial effect was similar to 1.5 percent garlic paste.

The anti bacterial efficacy of garlic in reducing TVC is reported by Yadav *et al (*2002) in chicken meat, *Staphylococcus aureus by* Kyung *et al.,* (2006), *Escherichia coli* by Sasaki *et al* (1999) and *Salmonella typhimurium* by Singh (2003) in chicken meat patties. In control sample, the counts linearly increased with increase in duration of storage without treatment. Similar findings have been recorded by Xavier and Baraquet (1994) with frankfurters and Gnanasambandam and Zayas (1994), who found a

Table 2: Effect of marinating with Garlic extract on Chicken meat spiked with reference strains on Microbial counts under refrigeration (8 ± 2 ^{0}C) (log10 cfu/g)

Time		Control	1.5 percent	2 percent
0hr	TVC	6.45 ± 0.11^a	6.39 ± 0.08^a	6.59 ± 0.06^a
	Escherichia coli	5.20 ± 0.03^a	5.29 ± 0.08^a	5.13 ± 0.03^a
	Staphylococcus aureus	4.68 ± 0.05^a	4.64 ± 0.05a	4.66 ± 0.04^a
	Salmonella typhimurium	3.99 ± 0.01^a	3.93 ± 0.10^a	3.97 ± 0.08^a
3 hr	TVC	6.71 ± 0.07^a	5.65 ± 0.12^b	5.54 ± 0.04^b
	Escherichia coli	5.48 ± 0.04^a	5.12 ± 0.06^b	4.55 ± 0.18^c
	Staphylococcus aureus	4.72 ± 0.05^a	4.07 ± 0.03^b	4.06 ± 0.04^b
	Salmonella typhimurium	3.86 ± 0.05^a	3.65 ± 0.06^b	3.43 ± 0.14^b
6 hr	TVC	6.81 ± 0.08^a	5.38 ± 0.06^b	5.19 ± 0.06^b
	Escherichia coli	5.70 ± 0.04^a	4.87 ± 0.06^b	4.37 ± 0.19^c
	Staphylococcus aureus	4.79 ± 0.05^a	3.83 ± 0.04^b	3.31 ± 0.05^c
	Salmonella typhimurium	4.20 ± 0.11^a	3.44 ± 0.03^b	3.07 ± 0.03^c
12 hr	TVC	6.85 ± 0.07^a	5.65 ± 0.07^b	5.26 ± 0.03^c
	Escherichia coli	5.77 ± 0.03^a	5.14 ± 0.07^b	4.76 ± 0.08^c
	Staphylococcus aureus	4.91 ± 0.03^a	4.09 ± 0.04^b	3.72 ± 0.02^c
	Salmonella typhimurium	4.35 ± 0.11^a	3.58 ± 0.04^b	3.29 ± 0.03^c
24 hr	TVC	7.18 ± 0.07^a	6.10 ± 0.02^b	5.71 ± 0.01^c
	Escherichia coli	5.85 ± 0.07^a	5.39 ± 0.07^b	5.23 ± 0.04^b
	Staphylococcus aureus	4.98 ± 0.02^a	4.43 ± 0.07^b	4.17 ± .03^c
	Salmonella typhimurium	4.52 ± 0.07^a	3.83 ± 0.03^b	3.67 ± 0.06^b
48hr	TVC	7.41 ± 0.12^a	6.40 ± 0.06^b	6.15 ± 0.09^b
	Escherichia coli	5.93 ± 0.07^a	5.44 ± 0.07^b	5.34 ± 0.04^b
	Staphylococcus aureus	5.08 ± 0.01^a	4.63 ± 0.09^b	4.48 ± 0.07^b
	Salmonella typhimurium	4.70 ± 0.07^a	4.01 ± 0.04^b	3.85 ± 0.09^b

BT: Before Treatment AT: After Treatment LR : Log Reduction
Means bearing different superscripts (a, b, c) within rows differ significantly (P< 0.01)

liner increase in total bacterial count with increase in duration.

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