

EFFECT OF ESSENTIAL OILS OF GARLIC, CLOVE AND CINNAMON ON *ESCHERICHIA COLI* IN CHICKEN MEAT NUGGETS

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ABSTRACT

Chicken meat nuggets were treated with essential oils of garlic and clove. Essential oils of garlic in treatment groups (T1 and T2) reduced the counts up to day 2 of storage afterwards there was a gradual increase in counts. Coliform count in chicken meat nuggets with essential oils of clove and cinnamon revealed that even though there was a progressive increase in counts up to day 14 of storage they were less in number when compared to the counts on day 0 of storage.

Key words: Essential oils of garlic, clove and cinnamon, chicken meat nuggets, *Escherichia coli*

INTRODUCTION

Since a long time garlic, clove and cinnamon have been used in the traditional Indian food preparations and further they are among the ten most inhibitory oils of spices (Deans and Ritchie, 1987) is often the most inhibitory to growth and survival of microorganisms (Draughon, 2004). Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties. Essential oils are a rich source of biologically active compounds.

There has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils. Hence the essential oils of spices or bio preservatives have the greatest advantage to cater to the demands of the consumer for the natural preservatives. Garlic, clove and cinnamon are some of the most commonly used ingredients as a flavour enhancer for foods. In addition to flavouring the foods, these spices are appreciated for their medicinal properties.

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Garlic, clove and cinnamon have a wide spectrum of actions not only antibacterial, anti viral, anti fungal and anti protozoal, but also have beneficial effects on the cardiovascular and immune system (Harris *et al.*, 2001). During the last decade the antimicrobial activity of garlic and garlic derived organosulfur compounds, eugenol and cinnamaldehyde (the antimicrobial compounds of clove) and cinnamon were widely investigated against both food spoilage bacteria and food borne pathogens (Naidu 2003, Unal *et al.*, 2001, Leuschner and Ielsch, 2003).

MATERIALS AND METHODS

Challenging studies of chicken meat nuggets with essential oils of garlic, clove and cinnamon were carried out with *Escherchia coli* (MTCC 452), as per the protocol of Fadia Naim *et al* (2004) with modifications. The reference strains of *Escherchia coli* (MTCC 452), was obtained from the Institute of Microbial Technology (IMTECH), Chandigarh.

Challenging with *Escherchia coli*

Escherchia coli (MTCC 452) strain was obtained as pure culture on agar slant and it was stored at 4°C. The culture was maintained at 4°C in Brain Heart Infusion broth and periodically tested for purity, morphology and biochemical characteristics. Stock cultures were prepared by streaking individual colonies on to MacConkey agar (OXOID) and incubated at 37 °C for 24 hours. Working cultures were prepared from daily consecutive transfer of *Escherchia coli* cells growing overnight at

37 °C in individual test tubes with Nutrient broth (HI-Media). The chicken meat mince which was free from coliform was inoculated with pure culture of *E. coli* diluted to a final concentration of 10⁹ cfu/ml. One millilitre of this bacterial suspension was mixed with the batter. Negative controls consisting of uninoculated samples were prepared. Both the inoculated and uninoculated samples were stored at a temperature of 5±1°C for evaluating the survival of the pathogen.

Coli form count

MacConkey agar was prepared and 1ml of inoculum of each dilution was placed in duplicate petridishes. The sterile molten (45 °C) MacConkey agar was poured in 10 to 15 ml quantities into each petridish and mixed thoroughly. The petridishes, after solidification of the medium were incubated at 37 °C for 18-24 hrs. Pink colored colonies were counted and expressed as log value /g of sample.

RESULTS AND DISCUSSION

Coliform count of chicken meat nuggets treated with essential oil of garlic at three different concentrations was significantly (P<0.05) different from control. Samples treated with essential oil of garlic at three different concentrations resulted in decreased coliform count up to day 4 of storage thereafter there was a gradual increase in coliform counts. Even though there was an increase in counts; they were well within the prescribed standards upto

14 days of storage (Table 1). The decreased coliform count when compared to control might be due to allicin, the antimicrobial component of garlic. The results of this study were similar to an earlier study of Johnson and Vaughn (1969) who reported reduction in the population of *Escherchia coli* inoculated into freshly reconstituted dehydrated garlic powder, Ceylan *et al.*, (1998) reported that garlic has also been shown to reduce *Escherchia coli* in ground meat, Kumar and Berewal (1998) reported that *Escherchia coli* was most sensitive to garlic Yadav *et al.*, (2002b) had reported a lower coliform count in garlic extract treated samples compared to the control, concomitantly, a significant reduction in coliform count was also reported in chicken meat mince treated with garlic extract by Yadav *et al.* (2002a), Sarma and Nandini (2004) reported inhibition of *Escherchia coli* in chicken legs which have been dipped in garlic extract for 10 minutes, Keily Alver De Moura Oliveira *et al.* (2005) found that the aqueous extract of garlic at 10 and 15 percent exhibited a reduction in coliform count and Muhammet Irfan Aksu *et al.*(2006) reported that garlic extract at 25 percent concentration inhibited the growth of *Escherchia coli* in 30 days.

With essential oil of clove, coliform count of chicken meat nuggets was significantly ($P<0.05$) different from control. Among the treatments, no significant ($P<0.05$) difference could be found between T1 and T2. Even though there was a gradual increase in coliform count

up to 14 day of storage, the counts were less than the counts recorded on day 0 of storage in all the treatments (Table2). The decreased coliform count when compared to control might be due to eugenol, the antimicrobial compound of clove. The results of this experiment were in agreement with Leuschner Renata and Zamparinijuliette (2002) who reported that clove oil at 0.25 to 1 percent showed bacteriostatic and bactericidal activities towards *E. coli* O157:H7. Sureenanasombat and Pana Lohasupthawee (2005) revealed that crude ethanolic extract of clove had most inhibitory action on the growth of *Escherchia coli*, Naveena *et al.*(2006) reported that clove oil with lactic acid and vitamin C had inhibitory effect on coliform count, Semaagaoglu *et al.*(2007) revealed that diethyl ether treated clove extract found to be most effective spice against *E. coli*. In chicken meat nuggets treated with essential oil of cinnamon, the *coliform* count was significantly ($P<0.05$) different from control. Even though there was a gradual increase in coliform count up to 14 days of storage, they were less than the counts registered on day 0 of storage in all the treatments (Table3). The decreased coliform count when compared to control might be due to cinnamaldehyde, the antimicrobial compound of cinnamon. The results obtained in this study were in association with Friedman *et al.* (2002) who reported that cinnamon oil had antimicrobial activity against *Escherchia coli in vitro*, Yadav *et al.* (2002b) had also reported a significant reduction in coliforms in chicken meat as well as in chicken

meat mince on addition of 8 percent cinnamon powder, Hoque *et al.*(2007) revealed that essential oil of cinnamon at 10 percent concentration had highest antimicrobial activity against *E.coli*. Charu Gupta *et al.* (2008) reported that cinnamon oil was found to be very effective against the same organism.

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Table 1: Mean ± SE values of coliform count of chicken meat nuggets with essential oil of garlic at refrigeration temperature (5 ± 1° C).

Storage Period In days	Control	Treatment with essential oil of garlic at a concentration of			Overall mean
		1 : 250	1 : 500	1 : 1000	
0	5.00 ± 0.08 ^{d3}	3.43 ± 0.15 ^{cd1}	3.29 ± 0.08 ^{cl}	4.08 ± 0.06 ^{b2}	3.95 ± 0.15 ^c
2	4.35 ± 0.10 ^{b4}	2.62 ± 0.15 ^{bl}	3.01 ± 0.05 ^{b2}	3.65 ± 0.14 ^{a3}	3.41 ± 0.15 ^b
4	4.12 ± 0.09 ^{a4}	2.27 ± 0.18 ^{al}	2.76 ± 0.13 ^{a2}	3.79 ± 0.17 ^{a3}	3.24 ± 0.17 ^a
6	4.81 ± 0.22 ^{c3}	3.32 ± 0.12 ^{cl}	3.58 ± 0.12 ^{dl}	4.19 ± 0.08 ^{bc2}	3.97 ± 0.14 ^c
8	5.03 ± 0.77 ^{d3}	3.49 ± 0.13 ^{dl}	3.61 ± 0.15 ^{dl}	4.28 ± 0.08 ^{c2}	4.10 ± 0.14 ^c
10	5.41 ± 0.07 ^{e4}	3.66 ± 0.14 ^{dl}	4.00 ± 0.08 ^{e2}	4.34 ± 0.03 ^{c3}	4.35 ± 0.14 ^d
12	5.73 ± 0.10 ^{f3}	4.09 ± 0.09 ^{el}	4.28 ± 0.10 ^{fl}	4.52 ± 0.04 ^{d2}	4.66 ± 0.14 ^e
14	5.79 ± 0.04 ^{f3}	4.12 ± 0.08 ^{el}	4.33 ± 0.03 ^{gl}	4.68 ± 0.03 ^{e2}	4.73 ± 0.14 ^e
Overall mean	5.03 ± 0.09 ^D	3.37 ± 0.10 ^A	3.61 ± 0.08 ^B	4.19 ± 0.06 ^C	

Note: Mean values bearing at least one common superscript / numerical with in each row do not differ significantly.

Mean values bearing at least one common superscript / numerical with in each column do not differ significantly.

Table 2: Mean \pm SE values of coliform count of chicken meat nuggets with essential oil of clove at refrigeration temperature ($5 \pm 1^{\circ}$ C).

Storage Period In days	Control	Treatment with essential oil of clove at a concentration of			Overall mean
		1 : 250	1 : 500	1 : 1000	
0	5.00 \pm 0.08 ^{d3}	4.61 \pm 0.08 ^{f1}	4.76 \pm 0.05 ^{e1}	4.86 \pm 0.21 ^{g2}	4.81 \pm 0.06 ^f
2	4.35 \pm 0.10 ^{b4}	2.28 \pm 0.08 ^{a1}	2.40 \pm 0.08 ^{a1}	2.88 \pm 0.09 ^{a2}	2.98 \pm 0.18 ^a
4	4.12 \pm 0.09 ^{a4}	3.07 \pm 0.05 ^{b1}	2.99 \pm 0.06 ^{b1}	3.00 \pm 0.06 ^{b1}	3.30 \pm 0.10 ^a
6	4.81 \pm 0.22 ^{c3}	3.09 \pm 0.05 ^{b1}	3.30 \pm 0.05 ^{b2}	3.59 \pm 0.16 ^{c3}	3.70 \pm 0.15 ^b
8	5.03 \pm 0.77 ^{d3}	2.92 \pm 0.04 ^{b1}	3.38 \pm 0.05 ^{b2}	3.54 \pm 0.12 ^{c2}	3.72 \pm 0.17 ^b
10	5.41 \pm 0.07 ^{e4}	3.91 \pm 0.04 ^{c1}	3.79 \pm 0.06 ^{c1}	4.08 \pm 0.09 ^{d2}	4.30 \pm 0.14 ^c
12	5.73 \pm 0.10 ^{f3}	4.09 \pm 0.08 ^{d2}	3.87 \pm 0.10 ^{c1}	4.26 \pm 0.10 ^{e3}	4.49 \pm 0.16 ^d
14	5.79 \pm 0.04 ^{f3}	4.23 \pm 0.08 ^{e1}	4.08 \pm 0.11 ^{d1}	4.54 \pm 0.09 ^{f2}	4.66 \pm 0.15 ^e
Overall mean	5.03 \pm 0.09 ^C	3.53 \pm 0.11 ^A	3.57 \pm 0.10 ^A	3.84 \pm 0.11 ^B	

Note: Mean values bearing at least one common superscript / numerical with in each row do not differ significantly.

Mean values bearing at least one common superscript / numerical with in each column do not differ significantly.

Table 3: Mean \pm SE values of *coliform* count of chicken meat nuggets with essential oil of cinnamon at refrigeration temperature ($5 \pm 1^{\circ}$ C).

Storage Period In days	Control	Treatment with essential oil of cinnamon at a concentration of			Overall mean
		1 : 250	1 : 500	1 : 1000	
0	5.00 \pm 0.08 ^{b3}	4.21 \pm 0.07 ^{d1}	4.49 \pm 0.07 ^{f1}	4.71 \pm 0.08 ^{f2}	4.60 \pm 0.07 ^e
2	4.35 \pm 0.10 ^{ab4}	2.03 \pm 0.07 ^{a1}	2.76 \pm 0.06 ^{c2}	2.31 \pm 0.07 ^{b3}	2.86 \pm 0.19 ^a
4	4.12 \pm 0.09 ^{a2}	2.14 \pm 0.05 ^{a1}	2.01 \pm 0.08 ^{a1}	2.01 \pm 0.06 ^{a1}	2.57 \pm 0.19 ^a
6	4.81 \pm 0.22 ^{b3}	2.10 \pm 0.08 ^{a1}	2.07 \pm 0.06 ^{a1}	2.58 \pm 0.07 ^{b2}	2.89 \pm 0.24 ^a
8	5.03 \pm 0.07 ^{b3}	2.44 \pm 0.13 ^{b1}	2.25 \pm 0.08 ^{b1}	3.09 \pm 0.06 ^{c2}	3.20 \pm 0.23 ^b
10	5.41 \pm 0.07 ^{c3}	2.68 \pm 0.07 ^{b1}	2.86 \pm 0.09 ^{cd1}	3.21 \pm 0.08 ^{c2}	3.54 \pm 0.23 ^c
12	5.73 \pm 0.10 ^{c3}	2.96 \pm 0.06 ^{b1}	2.94 \pm 0.06 ^{d1}	3.46 \pm 0.04 ^{d2}	3.77 \pm 0.24 ^d
14	5.79 \pm 0.04 ^{c3}	3.18 \pm 0.06 ^{c1}	3.16 \pm 0.11 ^{e1}	3.61 \pm 0.06 ^{e2}	3.93 \pm 0.23 ^d
Overall mean	5.03 \pm 0.09 ^D	2.72 \pm 0.10 ^A	2.82 \pm 0.11 ^B	3.12 \pm 0.12 ^C	

Note: Mean values bearing at least one common superscript / numerical with in each row do not differ significantly.

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