

Original Research Article

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Effect of Aqueous Extracts of Indian Spices on Inoculated *Listeria monocytogenes* in Chicken Breast Muscle

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ABSTRACT

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A study was carried out to determine the antimicrobial effect of aqueous extract of ginger, garlic and ginger-garlic in combination (1:2) on *Listeria monocytogenes* inoculated in chicken breast muscle. Inoculated breast muscles were subjected to dipping treatments and samples dipped in water were used as control. Results revealed that the mean *L. monocytogenes* count of water (control), ginger, ginger-garlic dipped samples were 4.35 ± 0.06 , 3.54 ± 0.03 , 3.58 ± 0.03 and 3.45 ± 0.03 , respectively. The mean log values of all the treated samples were significantly lower ($p \leq 0.05$) than that of the control. Among the treatment groups, ginger-garlic treated samples had significantly lower ($p \leq 0.05$) *L. monocytogenes* count than that of garlic treated samples whereas the count did not differ significantly between the former and ginger treated samples.

Introduction

In normal healthy animal, most of the tissues which ultimately become meat and meat products, including muscle, fat and various edible organs are sterile. During slaughter and processing all potential edible tissues are subjected to contamination from a variety of sources within and outside the animal (Ayes, 1995). Due to high nutrient content and poor hygienic conditions during handling, collection and processing, meat is considered to be highly perishable commodity. Owing to their nature and possessing favourable intrinsic factors, meat is frequently subject to

microbial contamination. Such contamination of meat is a major economic loss and may lead to public health problems when utilized as an ingredient in processed products.

In recent years, the epidemiology of foodborne diseases has been changing as new pathogens have emerged. Food borne diseases that are regarded as emerging include illness caused by enterohaemorrhagic *E. coli* (EHEC) or verocytotoxigenic *E. coli*, particularly serovar O 157:H7, *Camphylobacter jejuni*, *Listeria monocytogenes*, *Aeromonas spp.* and *Salmonella*, particularly serovar *Salmonella*

typhimurium DT 104. These pathogens have been frequently isolated from various meats. Among these micro-organisms, the epidemiology, pathogenesis and prevalence of *Listeria monocytogenes* have been studied by several authors (Drevets and Bronze, 2008; Ranjbar and Halaji, 2018; Jordan and McAuliffe, 2018; Tirumalai, 2013; Rajalakshmi *et al.*, 2017; Miraclin *et al.*, 2018; Suriyapriya *et al.*, 2016; Barbuddahe *et al.*, 2016). If the microbes on the surface of meat could be eliminated or substantially reduced immediately after slaughter, the risk of cross contamination during processing would be substantially reduced.

A variety of methods have been developed to reduce the level of contaminating bacteria on carcasses. The commonly used decontaminating agents include short chain organic acids, chlorine, hot water, certain polyphosphates and antimicrobial agents.

One such naturally occurring antimicrobial agent is spices. Spices have been defined as plant substances from indigenous or exotic origin used to enhance the flavour of foods. In addition to their contribution towards flavour, a wide variety of spices have been used for their antimicrobial property (Kumar and Jain, 2010; Rohani *et al.*, 2011). Keeping above points in view, the present study has been conducted to assess the effect of aqueous extracts of ginger, garlic and ginger-garlic combination on *L. monocytogenes* inoculated on chicken breast muscle.

Materials and Methods

Collection of samples

The chicken breast meat samples were collected from market in polyethylene bags and transported in insulated, refrigerated containers (4±1°C) to the Food and Industrial Microbiology Laboratory, College of Food

and Dairy Technology, Koduveli under hygienic conditions. Collected samples were portioned into 5 gram pieces and wrapped with aluminium foil. Then the wrapped samples were sterilized by autoclaving at 121°C for 15 min at 15 psi and were utilized for experimental inoculation studies.

Preparation of test strain

The standard serotype of *Listeria monocytogenes* (MTCC 657) was inoculated in BHI broth and grown overnight at 37° C. The cells were pelleted by centrifugation at 8000 rpm for 10 min and washed twice with sterile Phosphate Buffered Saline (PBS). The final cell pellet was suspended in PBS and the concentration of cells was adjusted to 10⁵ cells/ml using McFarland's nephelometer tubes.

Collection and preparation of extracts

The spices were procured from the local market. Aqueous extracts from garlic and ginger was prepared separately. The fresh garlic cloves and ginger rhizomes was washed, peeled, sliced and ground to obtain fresh extracts of the spices. To obtain aqueous extracts, the fresh extracts were diluted in 1:3 ratio using sterile distilled water. Aqueous extract of ginger-garlic combination was obtained by diluting each one part of fresh extracts of ginger and garlic with two parts of sterile distilled water (i.e., 1:1:2).

Inoculation in meat samples

Four sterile chicken breast muscle samples (each 5 g) was inoculated individually with *Listeria monocytogenes* (MTCC NO 657) organisms at a concentration of 10⁴ cells per gram of sample. After inoculation, the samples were kept for 20 min at room temperature to allow the bacteria to attach. All the procedures were carried out aseptically to avoid any contamination.

Treatment application

Among the four inoculated samples, one was dipped in 20 ml of sterile distilled water and kept as control. Other three samples were individually dipped in 20 ml of aqueous extracts of ginger, garlic and ginger-garlic combination. After immersion, all the samples including control were kept at room temperature for 45 min and then subjected to microbial analysis.

Microbial analysis

The inoculated samples were aseptically blended with 45 ml of 0.1% sterile peptone water in a stomacher. Decimal dilutions in sterile 0.1% peptone water were prepared from the blended samples, and one ml volumes were placed in duplicate onto sterilized petridishes. About 10 – 15 ml of sterile Listeria Selective Agar base (M1474; Himedia), added with rehydrated contents of Listeria Selective Supplement II (FD063), maintained at room temperature was poured in the inoculated petridishes and mixed thoroughly by rotating the plates. After solidification, plates were incubated at 37°C for 48±1 hrs. The number of colonies were multiplied by reciprocal of the dilution and expressed as log₁₀cfu/g of sample.

Statistical analysis

Data obtained from six trails were analysed

using standard statistical procedures (Snedecor and Cochran, 1994). One way Analysis of Variance (ANOVA) procedure was used to determine the significant difference (p<0.05) among means obtained for different treatments.

Results and Discussion

The effect of aqueous extracts of ginger, garlic and ginger-garlic combination on *Listeria monocytogenes* inoculated on chicken breast muscle have been presented in Table 1.

L. monocytogenes count

The overall mean *L. monocytogenes* count for control, ginger, garlic and ginger-garlic combination treated inoculated chicken breast muscle samples were 4.35±0.06, 3.58±0.03, 3.54±0.03 and 3.45±0.02, respectively.

The mean log values of all the treated samples were significantly lower than that of the control. Among the treatment groups, ginger-garlic combination treated samples had significantly lower (p≤0.05) count than that of ginger treated samples whereas the count did not differ significantly between the former and garlic treated samples. Overall, the aqueous extracts of ginger-garlic combination and garlic alone were equally effective in reducing the *L. monocytogenes* count.

Table.1 Effect of different treatments on Listerial count (log cfu/g of chicken breast muscle)

S.No.	Treatment groups	<i>L.monocytogenes</i> count (log ₁₀ cfu/g of sample)
1	Control	4.35±0.06
2	Ginger extract	3.58±0.03 ^b
3	Garlic extract	3.54±0.03 ^{a,b}
4	Ginger-garlic extract	3.45±0.02 ^a

Mean values within a column sharing same superscripts (a and b) did not differ significantly

Although the results showed all the treatments studied were effective in reducing the *L. monocytogenes* count compared to control, the reduction is only marginal. The mean log reduction in *L. monocytogenes* count in ginger, garlic and ginger-garlic combination treated chicken breast sample were only 0.77, 0.81 ± 0.03 , 0.9 ± 0.04 log cfu/g of sample, respectively. Result of the study is in concordance with that of several authors who studied anti-listerial effect of spices (Kumar and Berwal, 1998; Thongson, 2004; Indu *et al.*, 2006; Teimoory *et al.*, 2013). Indu *et al.*, 2006, during the study to assess the antimicrobial property of some South Indian species, found that the garlic and ginger extracts had minimum anti-listerial effect. Similarly, Teimoory *et al.*, (2013) also reported the minimum effect of ginger extract on *L. monocytogenes* during the study to assess its antimicrobial effect using agar diffusion technique. Kumar and Berwal (1998) also observed the minimum inhibitory effect of garlic on *L. monocytogenes*. Thongson *et al.*, (2004) also reported the reduced activity of garlic extract on *Listeria* and suggested that Gram positive organisms may be better equipped naturally to prevent the action of garlic extract.

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