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Antimicrobial Efficacy of Cardamom and Star Anise Powders on *E. coli* O157:H7 and other Microbial Quality Indicators in Minced Beef under Refrigeration Storage (5±1°C)

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ABSTRACT

Now-a-days consumers prefer natural foods without chemical preservatives. In this context, the present study was undertaken to evaluate antimicrobial efficacy of cardamom and star anise powders against E. coli O157:H7 and other common microbial indicators in minced beef during refrigeration storage (5±1oC). Reduction in E. coli O157:H7 population in 2 percent star anise powder treated samples was significantly (P<0.05) higher than samples treated with 2 percent cardamom powder and control samples. The standard plate count, coliform counts, psychrophilic and yeast and mold counts (log cfu/g) of treatment groups were significantly (P<0.001) lower than control throughout the storage period. Samples treated with 2 percent star anise powder showed significant (P<0.001) decrease in all microbial counts than 2 percent cardamom powder and control group. The addition of the 2 percent cardamom and 2 percent star anise powders into minced beef exhibited antimicrobial activity and successfully extended its shelf life to 5 and 6 days, respectively compared to 3 days in control samples at 5±1◦C.

Keywords: *Antimicrobial activity, Cardamom powder, E. coli O157:H7, Minced beef, Star anise powder*

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INTRODUCTION

The diverse nutrient composition of meat makes it an ideal medium for the growth and propagation of meat spoilage micro-organisms and common food-borne pathogens. Shiga toxigenic *E. coli* (STEC) is an increasingly important threat to public health worldwide due to consumption of undercooked beef products etc., which is mainly because of contamination of carcass during evisceration. It results in many human illness outbreaks such as haemorrhagic colitis, haemorrhagic uraemic syndrome, thrombocytic thrombocytopaenic purpura etc. *E. coli* O157:H7 serotype of STEC is mainly responsible for the illness especially among children and elders with less immunity.

Refrigerated ready to eat foods are becoming an important new class of products found in supermarket and convenience stores. It is, therefore, essential that adequate preservation technologies are applied to meat and meat products to maintain their safety and quality (Aymerich *et al.* 2008). Addition of antimicrobial chemical preservatives can better protect the meat from microorganisms. The growing concern about safety of foods has recently led to the development of natural antimicrobials to control food borne pathogens. Consumer demand is currently driven towards foods that are "natural" and free of additives. So the use of naturally occurring plant products for controlling food borne pathogen is considered to be a novel and acceptable means of securing safety of refrigerated food stuffs.

Plant-based essential oils or organic extracts and powders are well known to exhibit a wide range of antimicrobial and antioxidant effects (Devatkal *et al.* 2011 and Najeeb *et al.* 2015). Spices and essential oils are used by the food industry as natural agents for extending the shelf life of foods. The main commercial objective of adding these compounds to foodstuff is extending their shelf-life and increasing, if possible, their nutritional and organoleptic value (Silvério and Lopes, 2012).

Cardamom (*Elettaria cardamomum*) known as "queen of spices" belongs to the family *Zingiberaceae*. The basic cardamom aroma is produced by a combination of the major components, 1, 8-cineole and α-terpinyl acetate (Lawrence, 1979). The seeds and essential oil are used as flavouring components in a variety of foods. It is used as a powerful aromatic, carminative, diuretic and stimulant in ayurvedic medicine. Cardamom possesses antibacterial, antifungal (Agaoglu *et al.* 2005; Bansod and Rai 2008; Singh *et al.* 2008), anticancer (Sengupta *et al.* 2005), antioxidant (Singh *et al.* 2008; Lin *et al.* 2009; Sultana *et al.* 2010) and also gastro-protective effects (Jamal *et al.* 2006).

Star anise (*Illicium verum*), belongs to the family *Illiciaceae*. It has been widely used in traditional medicine for their antimicrobial effects. The most abundant component present in star anise is trans-anethole (89.5 percent) (Huang *et al.* 2010). Star anise oil has got potent antifungal properties (Dzamic *et al.* 2009). Volatile oils from star-anise has antioxidant (Padmashree *et al.* (2007) and antimicrobial (Singh *et al.* 2007) activities. Yazdani *et al.* (2009) recorded the inhibition of growth of all dermatophytes and saprophytes by extracts of star anise fruits in vitro. Madhumita and Ramalingam (2010) reported that both aqueous and ethanolic extracts of star anise were found to possess broad spectrum antimicrobial activity.

Nowadays spices are used as natural preservatives and based on available literature it has been observed that cardamom and star anise possess antimicrobial activity, but reports on their use in meat as a natural antimicrobial agent individually are scanty. In the present work, the antimicrobial efficacy of 2 percent cardamom and

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2 percent star anise powder was evaluated against *E. coli* O157:H7 and other common microbial indicators in minced beef during its refrigeration storage (5±1°C).

MATERIALS AND METHODS

Beef sample: Fresh beef was procured from a hygienic retail outlet of Puducherry and was brought to the laboratory within 1 hour maintaining cold chain. It was then thoroughly washed and cleaned, cut into smaller chunks and minced through 8mm plate in a meat mincer (Mado Shop Mincer Junior, Germany) in the Department of Livestock Products Technology, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER).

Evaluation of antimicrobial efficacy of cardamom and star anise powders on E. coli O157:H7 in minced beef under refrigeration storage (5±1°C): Challenge studies of the minced beef treated with two percent cardamom and two percent star anise powder were carried out with *E. coli* O157:H7 as per the protocol of Fadia Naim *et al.* (2004). The reference strain of *E. coli* O157:H7 obtained from Department of Animal Biotechnology, Madras Veterinary College, Chennai and maintained in the Department of Veterinary Microbiology, RIVER was used for the study. The reference culture was streaked on Sorbitol Mac Conkey's agar and incubated at 37°C for 24 hours. A single colony transferred into Luria broth and incubated at 37°C for 18 hours was used as working culture. The concentration of the cells in the broth was enumerated by pour plate method using Sorbitol Mac Conkey's agar.

O157:H7 culture was diluted to a final concentration of 108 cells /ml. The minced beef was inoculated with working culture of these reference strains. One ml of each bacterial suspension was thoroughly mixed in 100 gram of beef. The cardamom and star anise powder was added at the rate of two per cent (w/w) levels. A control beef sample containing inoculated bacterial culture was maintained without the addition of the powders. The samples were packed in LDPE bags and stored at refrigeration temperature (5±1°C) up to 6 days. Evaluation of the survival of the pathogens was done on alternate days up to six days by pour plate method.

Preparation of serial dilutions: Ten grams of samples were weighed aseptically and transferred to a sterile mortar to make 10^{-1} dilution, then serial dilutions were made. Preparation of samples and serial dilutions were done near flame in a horizontal laminar air flow observing all possible aseptic conditions.

Sorbitol Mac Conkey's agar (SMAC) was used for the enumeration of *E. coli* O157:H7. The media was prepared and 1 ml of each dilution was placed in duplicate petridishes. The sterile, molten and cooled (45°C) SMAC agar was poured in about 15 ml quantities in each of the petridishes separately and thoroughly mixed. After solidification of the media, the petridishes were incubated in inverted positions at 37°C for 18 to 24 hours. Based on colony characters, the colonies were differentiated for counting and were subsequently expressed as log cfu/gram of sample.

Evaluation of antimicrobial efficacy of cardamom and star anise powders on common microbial indicators in minced beef under refrigeration storage (5±1°C): The cardamom and star anise powder were added to the minced beef at the rate of two percent (w/w) levels. A control was maintained without the addition of the powders. Then it was packed in LDPE bags and stored under refrigeration (5±1°C). Evaluation of its microbiological quality was done by means of standard plate count (SPC), coliforms, psychrophilic, and yeast and mold counts (YMC) on 0, 2, 4, 6, 7 and 8th day of storage.

Microbial quality analysis: Microbial parameters viz, standard plate count (SPC), coliform count, psychrophilic count and yeast and mold counts (Y&M) of minced beef were determined following procedures recommended by APHA (1984). Plate count agar (HiMedia, Mumbai) for standard plate count and psychrophilic count, brilliant green agar (HiMedia, Mumbai) for coliform count and potato dextrose agar (HiMedia, Mumbai) for yeast and mold count were used.

Statistical analysis: Each experiment was repeated thrice and each parameter was analyzed in duplicate. The data were analyzed using SPSS version 16.0 MSI (SPSS, Chicago, USA). Two way analysis of variance (ANOVA) was applied for storage study. The level of significance was tested using the least significant difference (LSD) test (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

Antimicrobial efficacy of the cardamom and star anise powder on Escherichia coli O157:H7 in minced beef under refrigeration (5±1°C): Cardamom and star anise powders each at 2 percent level did not exert any immediate antimicrobial effect on 0 day (Table 1). But on 2nd day of storage at 5±1°C, a significant ($p<0.05$) reduction in counts of *E. coli* O157:H7 were observed in both the treated samples compared to control. Star anise powder showed bactericidal activity against *E. coli* O157:H7 resulting in significant ($p<0.05$) reduction in counts from log 6.51 on 0 day to log 4.63 on 2nd day. On 4th day counts increased non-significantly to 5.80 and then to log 6.41 on 6th day which was significantly ($p<0.05$) higher than the counts on 2nd day but almost similar to counts on 0 day.

A significant reduction (log 0.8 and log 1.7) in *E. coli* O157: H7 was observed on 6th day in cardamom and star anise treated samples. Results revealed that both cardamom and star anise powders had antibacterial effect on *E. coli* O157: H7 with star anise powder having a higher bactericidal activity than cardamom powder. The results are in accordance with the findings of Singh *et al.* (2007) who reported that volatile oil of star anise was found to be highly active against *E. coli.* Kandasamy *et al.* (2011) reported that crude alcoholic extracts of cardamom was less active against *E. coli.* Madhumita and Ramalingam (2010) reported that aqueous and ethanolic extracts of both cardamom and star anise showed same antimicrobial effect against *E. coli.* Agaoglu (2005) reported

that seed extracts of cardamom produced the least inhibitory effect on *E. coli*, whereas, S. aureus was detected to be the most sensitive bacteria.

Antimicrobial efficacy of the cardamom and star anise powder on common microbial indicators in minced beef under refrigeration (5±1°C)

Standard plate count (SPC): The SPC (log cfu/g) in control, 2 percent cardamom and 2 percent star anise powder treated groups ranged between 4.48 - 10.34, 4.42 - 9.60 and 4.39 - 9.12, respectively for 0 day to 8th day of storage (Table 2).

Table 1: Antimicrobial efficacy of cardamom and star anise powder against E. coli O157:H7 (log cfu/g) in minced beef under refrigeration (5±1°C) (Mean ±SE).

Storage period	Control	2 percent Cardamom	2 percent Star anise
0 day	6.97 ± 0.44^{aA}	6.76 ± 0.40^{aA}	6.51 ± 0.44^{bA}
2 days	7.25 ± 0.31^{abC}	6.14 ± 0.38^{aB}	4.63 ± 0.24^{aA}
4 days	7.64 ± 0.32^{abB}	6.56 ± 0.37^{aAB}	5.80 ± 0.44^{abA}
6 days	8.14 ± 0.13^{bC}	7.33 ± 0.18^{aB}	6.41 ± 0.27^{bA}

Means with different superscripts (capital letters in the same row and small letters in the same column) differ significantly (p<0.05)

The SPC of all the treatment groups were significantly (p<0.001) lower than that of control on all days except on 0 day. All the groups revealed a gradual and significant (p<0.001) increase in counts with increase in storage days except on 2nd day, where both the treated groups had similar counts as on 0 day. The counts in all the treated groups differed significantly (p<0.001) from control and from each other on all the days of storage. Among the treatment groups, star anise treated group showed significantly (p<0.001) lower counts than cardamom treated group. However, counts in the treated groups remained significantly (p<0.001) lower than control until 8th day of storage. However, by the 7th day of storage, SPC of star anise powder treated samples (7.17) exceeded the maximal recommended limit of 7 (log cfu/g) for SPC in raw meat (ICMSF, 1986), indicating a shelf life of about 6 days under refrigeration storage (5±1°C). The lower microbial counts in treated samples might be due to presence of antimicrobial compounds such as 1, 8-cineole (20-60 percent) and α-terpinyl acetate (20-53 percent) in cardamom (Nanasombat *et al.* 2005) and *trans*-anethole (89.5 percent) in star anise (Huang *et al.* 2010). Similar to these findings, Shekhar *et al.* (2011) reported that chicken legs spiked with reference strains of *E. coli*, *Staphylococcus aureus*, Salmonella and marinated with 2 percent clove powder had slower rate of proliferation of total viable counts, *E. coli*, *Staphylococcus* counts and *Salmonella* counts than 1 percent clove powder treated samples under refrigeration.

Coliform count: The coliform count (log cfu/g) for control, 2 percent cardamom and 2 percent star anise powder treated groups ranged between 3.24 – 9.46, 3.13 – 8.89 and 2.96-8.12, respectively for 0 day to 8th day of storage (Table 3). Both treatment groups contained significantly (p<0.001) lower coliform count than control on 0 day. Though the groups showed a gradual and significant (p<0.001) increase in counts from day 0 to 8, the values for both treated groups remained significantly (p<0.001) lower than control throughout the storage period.

Table 2: Effect of cardamom and star anise powder on SPC (log cfu/g) in minced beef stored under refrigeration (5±1°C) (Mean ±SE).

Storage period (Days)	Control	2 percent Cardamom	2 percent Star anise
0	4.48 ± 0.12^{aA}	4.42 ± 0.11^{aA}	4.39 ± 0.11^{aA}
2	5.20 ± 0.09^{bB}	4.48 ± 0.12^{aA}	4.38 ± 0.13^{aA}
4	6.38 ± 0.12^{cC}	5.27 ± 0.04^{bB}	4.95 ± 0.05^{bA}
6	7.55 ± 0.16^{dC}	6.78 ± 0.09^{cB}	6.32 ± 0.12^{cA}
7	8.52 ± 0.15^{eC}	7.88 ± 0.05^{dB}	7.17 ± 0.05^{dA}
8	10.34 ± 0.10^{fB}	9.60 ± 0.23^{eA}	9.12 ± 0.09^{eA}

Means with different superscripts (capital letters in the same row and small letters in the same column) differ significantly (p<0.001)

Coliform counts in both the treated groups differed significantly (p<0.001) from control and from each other on all the days of storage. However, star anise treated group showed the lowest count compared to cardamom treated and control samples. The observations recorded in the present study are similar to the results reported by Agaoglu, (2005); Singh *et al.* (2007); Madhumita and Ramalingam, (2010); Kandasamy *et al.* (2011) who observed that star anise was comparatively more effective than cardamom in inhibiting coliform bacteria in in-vitro studies.

Table 3: Effect of cardamom and star anise powder on the coliform counts (log cfu/g) in minced beef stored under refrigeration (5±1°C) (Mean±SE)

Storage period (Days)	Control	2 percent Cardamom	2 percent Star anise
0	3.24 ± 0.06^{aB}	3.13 ± 0.06^{aAB}	2.96 ± 0.08^{aA}
2	3.99 ± 0.29^{bC}	3.66 ± 0.29^{bB}	3.28 ± 0.12^{aA}
4	6.07 ± 0.12^{cC}	4.78 ± 0.11^{cB}	4.17 ± 0.12^{bA}
6	7.27 ± 0.15^{dB}	6.07 ± 0.04^{dA}	5.49 ± 0.25^{cA}
7	8.20 ± 0.04^{eB}	7.02 ± 0.57^{eA}	6.65 ± 0.20^{dA}
8	9.46 ± 0.09^{fC}	8.89 ± 0.48^{fB}	8.12 ± 0.04^{eA}

Means with different superscripts (capital letters in the same row and small letters in the same column) differ significantly (p<0.001).

Psychrophilic count: The psychrophilic counts (log cfu/g) in control, 2 percent cardamom and star anise powder treated groups ranged between 4.09-10.74, 4.04-10.44 and 4.02-9.99, respectively for 0 day to 8*th* day of storage (Table 4). All the groups revealed a gradual and significant (p<0.001) increase in counts with increase in storage days. Among the treatment groups, star anise treated group showed significantly (p<0.001) lower counts than cardamom treated group. However, psychrophilic counts in both the treated

groups remained significantly ($p<0.001$) lower than control till 8th day of storage. Natural preservatives such as pomegranate fruit juice phenolic solution showed antimicrobial activity against psychrotrophic bacteria in chicken meat (Vaithiyanathan *et al.* 2011). Sallam and Samejima (2004) reported that the initial psychrotrophic count in ground beef samples ranged from 4.0 to 4.14 (log cfu/g), with control being the highest at day 21 (9.98 log cfu/g) followed by samples treated with sodium chloride (8.87 log cfu/g), while much lower counts were obtained in samples treated with sodium lactate either alone (7.79 log cfu/g) or in combination with sodium chloride (6.44 log cfu/g).

Table 4: Effect of cardamom and star anise powder on the psychrophilic counts (log cfu/g) in minced beef stored under refrigeration (5±1°C) (Mean ±SE)

Storage period (Days)	Control	2 percent Cardamom	2 percent Star anise
0	4.09 ± 0.17^{aA}	4.04 ± 0.17^{aA}	4.02 ± 0.18^{aA}
2	4.89 ± 0.29^{bB}	4.26 ± 0.08^{aA}	4.11 ± 0.04^{aA}
4	7.26 ± 0.05^{cC}	5.12 ± 0.06^{bB}	4.64 ± 0.06^{bA}
	7.81 ± 0.18^{dC}	6.73 ± 0.15^{cB}	6.13 ± 0.02^{cA}
	9.12 ± 0.09^{eC}	7.60 ± 0.12^{dB}	7.02 ± 0.15^{dA}
	10.74 ± 0.11^{fB}	10.44 ± 0.07^{eAB}	9.99 ± 0.20^{eA}

Means with different superscripts (capital letters in the same row and small letters in the same column) differ significantly ($p<0.001$)

Yeast and mold count: Yeast and mold counts (log cfu/g) in control, 2 percent cardamom and star anise powder treated groups ranged between 2.52 - 6.59, 2.30 - 6.04 and 2.05 - 5.18, respectively, for 0 day to 8th day of storage (Table 5). Yeast and mold counts of the treatment groups were significantly ($p<0.05$) lower than control on all days of storage except on 2nd day. Though the groups showed a gradual and significant ($p<0.05$) increase in counts from day 0 to 8, the values for star anise powder treated group remained significantly ($p<0.05$) lower than cardamom powder treated and control samples. No significant differences were observed between 2 percent cardamom powder treated and control samples on all the days of storage. Similar to these findings, Yazdani *et al.* (2009) recorded the inhibition of growth of all dermatophytes and saprophytes by extracts of star anise fruits. Huang *et al.* (2010) reported that the fruit essential oil of I. *verum* and trans-anethole had a wide inhibitory spectrum of activity against plant pathogenic fungi. Silvério and Lopes (2012) observed that star anise, clove and allspice completely inhibited the growth of three different *Aspergillus* species (*Aspergillus ochraceus, Aspergillus versicolor* and *Aspergillus flavus*) and also inhibited their toxin production. Hema *et al.* (2009) reported that 50 percent concentration of alcoholic extract of cardamom showed inhibitory action against *Aspergillus niger*. Kandasamy *et al.* (2011) in their antimicrobial study found that cardamom was highly active against *Aspergillus niger*. Aneja *et al.* (2009) reported that cardamom extracts were effective against oral pathogenic bacteria like Streptococcus mutans and *Candida albicans*.

Table 5: Effect of cardamom and star anise powder on the yeast and mold counts (log cfu/g) in minced beef stored under refrigeration (5±1°C) (Mean ±SE)

Storage period (Days)	Control	2 percent Cardamom	2 percent Star anise
0	2.52 ± 0.15^{aB}	2.30 ± 0.15^{aAB}	2.05 ± 0.07^{aA}
2	2.84 ± 0.28^{aA}	2.69 ± 0.34^{aA}	2.20 ± 0.15^{abA}
4	3.82 ± 0.22^{bB}	3.43 ± 0.24^{bB}	2.50 ± 0.17^{bA}
6	4.87 ± 0.17^{cB}	4.60 ± 0.08^{cB}	3.81 ± 0.11^{cA}
7	5.70 ± 0.26^{dB}	5.45 ± 0.25^{dAB}	4.78 ± 0.11^{dA}
8	6.59 ± 0.11^{eC}	6.04 ± 0.08^{dB}	5.18 ± 0.10^{eA}

Means with different superscripts (capital letters in the same row and small letters in the same column) differ significantly ($p<0.05$)

CONCLUSIONS

Both the 2 percent cardamom and 2 percent star anise powders possessed significant inhibitory effect on *E. coli* O157:H7, SPC, coliforms, psychrophilic, yeast and mold counts in minced beef under refrigeration. The shelf life of control, 2 percent cardamom and 2 percent star anise powders treated minced beef under refrigeration (5±1°C) storage were 3, 5 and 6 days, respectively and thus 2 percent star anise powder exhibited better antimicrobial activity compared to 2 percent cardamom powder.

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ETHICS STATEMENT: Not Applicable

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