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Sanitizing effect of lemon grass oil and citrus fruit extract (Orange peel powder and lemon juice) in meat industry

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Abstract
Foodborne diseases are considered as one of the serious global health problems. Contamination of food from animal origin could occur at any stage of production process till it reaches the consumer. Cutting board is one of the top five sites most contaminated with heterotrophic bacteria and may facilitate transmission of foodborne pathogens by cross- contamination. Nowadays, the meat industry is facing increasing pressure from the consumers to reduce the use of chemical components in meat contact surfaces. In this context, the present study was conducted at Meat Technology Unit, College of Veterinary and Animal Sciences, Mannuthy, to compare the sanitizing effects of lemon grass oil and citrus fruit extracts (orange peel powder and lemon juice) in meat cutting boards and thus to replace chemical sanitizers used for disinfection of meat cutting boards. Swab samples were collected from the meat cutting boards of Cattle slaughter hall of Meat Technology Unit. Without the application of any sanitizing agents (Control) and 30 minutes after the application of lemon grass oil, lemon juice and orange peel extract. The surface swab samples collected were used for the enumeration of Total viable count (TVC), coliform count and Yeast and Mould count. Among all the three treatments, lemon grass oil (T2) resulted in significant reduction (p<0.05) in the mean values of TVC, coliform count and yeast and mould count when compared to control. Lemon grass oil can be used as effective alternative against hazardous chemical sanitizers used in meat industry without affecting the cost.

Keywords: Lemon grass oil, citrus fruit extract, meat industry

Introduction
Foodborne diseases are considered as one of the serious global health problems. Foodborne diseases cause serious public health issues leading to morbidity and mortality, and a significant impediment to socioeconomic development worldwide. Contamination of food from animal origin could occur at any stage of production process till it reaches the consumer. The causative agents of foodborne diseases involves bacterial agents, parasites, viruses, prions, metals and toxins. Food safety refers to the ways in which food is prepared, cooked, chilled, served and overall handled. It is during these processes that improper food handling or a lack of food knowledge can lead to the spread of dangerous germs, bacteria, and allergens through food.

The commitment to food preparation at homes decreased and number of meals eaten out of the home has increased. Consumers favor convenience and saving time rather than proper handling and preparation of food. Microbial contamination and foodborne illnesses through hotel food are on the rise in Kerala.

Meat cutting is done in the home, restaurants and meat processing facilities. The study of microbial contamination of meat chopping boards must be differently apprehended according to the situation considered. Cutting board is one of the sites most contaminated with heterotrophic bacteria and may facilitate transmission of foodborne pathogens by cross-contamination. Residues from raw meat or poultry might remain on the work surface and transfer disease agents. Some of the bacteria-though not viruses or other disease agents can multiply on the surface between being deposited from the first food and contaminating another. Microorganisms that gets deposited in the cracks of meat cutting board surface can come into contact with meat when the meat is chopped. Hence the use of sanitizers has become essential for the prevention of food borne illnesses originating from the meat cutting boards.

There are many interventions which can eliminate microbial load on meat contact surfaces which includes hot or cold water washing, spraying of steam or hot water etc.

Organic acids such as acetic acids, lactic acids or propionic acids spray are considered as much effective disinfectants. Chemicals such as chlorine, acidified sodium chlorite, peroxyacids, and trisodium phosphate were also used to reduce the microbial load in cutting surfaces.

Nowadays, the meat industry is facing increasing pressure from the consumers to reduce the use of chemical components in meat contact surfaces. This explains the growing tendency to use natural plant-based or plant-derived substances that can act as antimicrobial agents and protect food from further microbial contaminations. Consumer demand for natural preservatives has increased rapidly, whereas the safety aspect of chemical additives has been questioned. Essential oils and extracts obtained from many plants have recently gained a great popularity and scientific interest. The antimicrobial activity of essential oils from oregano, thyme, sage, rosemary, clove, coriander, cinnamon, garlic and onion against food-related microorganisms as well as their applications in food system have been investigated.

Materials and methods

The present study was undertaken with the objective of comparing the effectiveness of lemon grass oil and citrus fruit extracts (orange peel powder and lemon juice) as sanitizing agents on meat cutting boards. The study was conducted at Meat Technology Unit, College of Veterinary and Animal Sciences, Mannuthy.

Collection of Samples

Swab samples were collected from the meat cutting boards of cattle slaughter hall of Meat Technology Unit, Mannuthy, Thrissur. The samples were collected using sterile cotton swab with screw cap (Hi-Media, Mumbai).The swab handle was held at 30 angle contact with the table surface. An area of 100 square centimeters of the table was marked with sterile aluminum frame of 10 cm x 10 cm and rubbed the swab head slowly and thoroughly over the surface using parallel strokes reversing direction between strokes and rotation of the swab continuously for 30 sec, these swabs were transferred to a screw capped test tube. The swabs were taken without the application of any sanitizing agents (Control) and 30 min after the application of lemon grass oil, lemon juice and orange peel extract.

Processing of Samples

The collected samples were processed in the laboratory facility available in the Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy. Processing of swab samples was carried out with maximum aseptic precautions. The screw cap plastic tubes of the swabs were removed carefully and transferred to test tubes containing 0.1 percent peptone water and shaken for 30 sec for uniform distribution of the microorganisms into the solution.

Preparation of Chemicals

Preparation of peptone water

Peptone water was prepared for serial dilution of sample. Fifteen gram of 0.1 percent peptone water powder (hi-media M1748, Mumbai) in 1,000 L of distilled water was mixed thoroughly, sterilized by autoclaving at 1210C and 15 pounds pressure for 15 min. The final pH of the medium was adjusted to 7.0 ± 0.02 at 250

Serial dilution

All the samples were brought to the laminar air flow chamber (Rotek, Mumbai) which was pre-sterilized by ultra-violet radiation for 20 min before use. One mL of homogenised sample solution was transferred to nine mL of sterile peptone water in a test tube and mixed uniformly to get 10-1 dilution. One mL of 10-1 dilution was added to nine milliliter of peptone water and mixed to obtain 10-2 dilution and so on until serial dilution was made as per requirement.

Preparation of Natural Extracts

Five percent solutions of lemon juice (T1) and lemon grass oil (T2) were prepared by mixing five mL of lemon juice and lemon grass oil in 100 mL of distilled water. Five percent solution of orange rind was prepared by mixing and heating five gram of dried and powdered orange peel in 100 mL of distilled water.

Table 1: Treatment of meat cutting boards from cattle slaughter hall with lemon grass oil, lemon juice and orange peel extract were given as follows

Group	Treatment
C	CONTROL (untreated cutting board)
T1	Five percent solution of lemon juice
T2	Five percent solution of lemon grass oil
T3	Five percent solution of orange peel extract

Microbial Counts

Total Plate Count

Total Plate count (TPC) of each sample was estimated by pour plate technique, as described by Morton (2001). In 1,000 mL of distilled water, 30 g of plate count agar (Hi- media, Mumbai) was suspended, boiled to dissolve the agar completely and sterilized by autoclaving at 121ºC and 15 pounds pressure for 15 min. The final pH of the medium was adjusted to 7.0 ± 0.02 at 25ºC. From the selected tenfold dilution of each sample, one millilitre of the inoculum was transferred on to duplicate petri-dish of uniform size. To each of the inoculated plates about 10 to 15 mL sterile molten standard plate count agar (Hi-media m091) maintained at 45ºC was poured and mixed with the inoculum by gentle rotary movement i.e., clockwise, anticlockwise, forward and backward. The inoculated plates were left at room temperature and allowed to solidify and were incubated by inverting the plates and maintained at 37oC for 24 h. At the end of incubation, plates showing colony forming units (cfu) between 30 and 300 colonies were selected and were counted with the help of a colony counter. The number of cfu per sample was calculated by multiplying the mean count in duplicate plates with the dilution factor and was expressed as log10 cfu/cm^2.

Coliform Count

In order to estimate the coliform count from the processed sample, the media was prepared first by suspending 41.3 g in 1,000 mL of distilled water, 41.53 g of violet red bile agar (Hi-Media M049, Mumbai) was suspended, boiled to dissolve the agar completely, and sterilized by autoclaving at 121oC and 15 pounds pressure for 15 min. The final pH of the medium was adjusted to 7.4 ± 0.02 at 25oC. From the selected tenfold dilution of each sample, one milliliter of the inoculum was transferred on to duplicate petri-dish of uniform size. To each of the inoculated plates about 10 to 15 mL sterile molten

agar (Hi-Media M049) maintained at 45ºC was poured and mixed with the inoculum by gentle rotary movement i.e., clockwise, anticlockwise, forward and backward. The inoculated plates were left at room temperature and allowed to solidify, and incubated by inverting the plates and maintained at 37oC for 24 h. At the end of incubation, plates showing between 30 to 300 colonies were selected and were counted taken with the help of a colony counter. The number of colony forming units (cfu) per sample was calculated by multiplying the mean count in duplicate plates with the dilution factor and was expressed as log 10 cfu/cm^2.

Yeast and Mould Count

The method described by Beuchat and Cousine (2001) [4] was followed for estimation of yeast and mould count. Potato dextrose agar (Hi-Media M096) was used and count estimated by spread plate technique. In 1,000 mL distilled water, 39 g of potato dextrose agar (Hi- Media M096, Mumbai) was suspended, boiled to dissolve the agar completely and sterilized by autoclaving at 1210 C and 15 lb pressure for 15 min. To obtain a pH of 3.5, the sterilized cooled medium was acidified with 10 mL of 10 percent sterile tartaric acid. Precaution was taken not to heat the medium after addition of the acid to preserve solidifying properties of agar. About 10-15 mL of melted agar, maintained at 44- 46oC was poured gently into each petri-dish. The agar was allowed to solidify and the plates were kept in inverted position in an incubator maintained at 35oC for 12 h. From the selected dilution of each sample, 0.1 mL of inoculum was transferred on to duplicate plates containing the media and the inoculum was evenly distributed on the media with a sterile 'L' shaped glass rod. The plates were incubated at 25oC for three to five days. After the period of incubation the black, white, red and green colored colonies appeared on the plates were counted with the help of colony counter. Multiplied by dilution factor and expressed as log10 cfu/cm^2 per sample.

Result and Discussion

The results of microbiological quality evaluations of C-control (Untreated cutting board sample), T1-treatment with lemon for 30 min, T2- treatment with lemon grass oil for 30 minutes, and T3- treatment with orange peel extract for 30 min are presented as follows.

Table 2: Microbiological quality (Mean ± SE, log10 cfu/cm^2) of meat cutting board sanitized with different treatment solutions.

Microbiological quality	C	T1	T2	T3	F value	P value
Total viable count (log10 cfu/cm^2)	5.21±.02 a	4.54±.25 b	4.36±.03 b	4.93±.01 a	8.610**	<0.001
Coliform Count (log10 cfu/cm^2)	4.18 ±.04a	3.99±.04b	3.31±.06c	4.06±.03a,b	78.961**	<0.001
Yeast and mould count (log10 cfu/cm^2)	2.59 ±.09a	2.48±.03a	2.16±.04b	2.54±.05a	10.549**	<0.001

C-control (Untreated cutting board sample), T1-Treatment with lemon for 30 min, T2- Treatment with lemon grass oil for 30 min, T3- Treatment with orange peel extract for 30 min

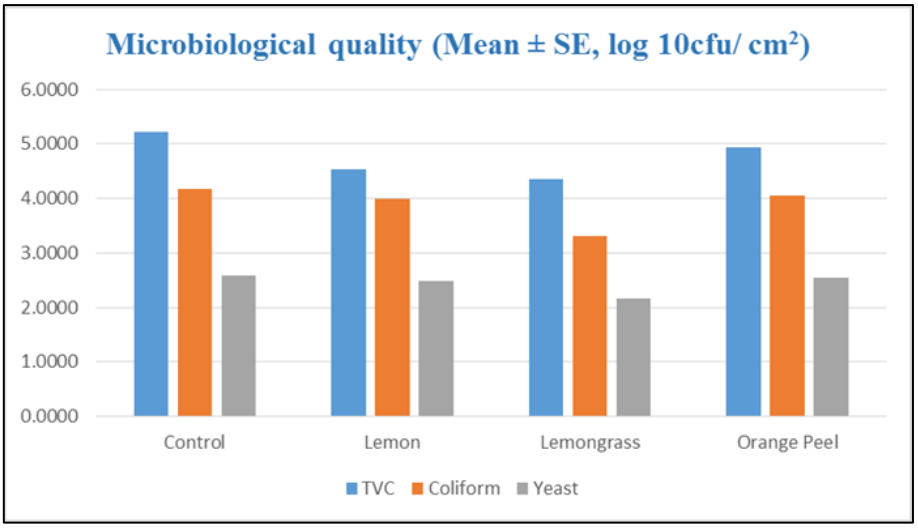

Fig 1: Microbiological quality (Mean ± SE, log 10cfu/ cm^2) of meat cutting board sanitized with different treatment solutions.

C-control (Untreated cutting board sample), T1-Treatment with lemon for 30 min, T2- Treatment with lemon grass oil for 30 min, T3- Treatment with orange peel extract for 30 min.

The total viable count (TVC) of the meat cutting boards treated with lemon, lemon grass oil and orange peel extract for 30 min (T1, T2, and T3) with those of control(C) (Untreated meat cutting board) are presented in Table 2 and Figure 1. The mean TVC (log10cfu/cm^2) of untreated cutting board (control) was 5.21± 0.02. The mean TVC of the meat cutting boards treated with lemon, lemon grass oil and orange peel extract for 30 min

i.e. T1, T2 and T3 were 4.54±0.25, 4.36±0.03 and 4.93±0.01 respectively. The mean values of the total viable count (TVC) of control differ significantly (p<0.05) from that of T1 (lemon juice) and T2 (lemon grass oil). The lowest TVC was observed in T2 whereas the highest TVC was observed in the untreated meat cutting board (control).

Total viable count (TVC) describes a measure of bacteria in the sample that can survive in the conditions on the surfaces or carcasses or in processed meat, be harvested by the sampling procedure used and grow in the presence of air on an agar plate. These bacteria include those arising both from animals and from the slaughterhouse or meat processing environment. Bacterial contaminants are diverse and include non-pathogenic and pathogenic taxa. As the TVC includes the organisms responsible for spoilage of meat, it will also give an indication of the keeping quality of the meat. The observed

results in this experiment indicate the reduction in the mean value of TVC is due to the strong antimicrobial activity of the lemon grass oil. There was 0.85 log10cfu/cm^2 reduction in the mean value of TVC in the cutting board surface treated with lemon grass oil when compared with untreated cutting board (control) sample.

The mean value of coliform count of Coliforms including *E. coli* have been implicated in several meat borne disease outbreaks around the world. The mean value coliform count (log cfu/cm^2) of control (untreated) meat cutting board sample was 4.18±0.04. The mean coliform count (log10cfu/cm^2) of meat cutting boards treated with lemon, lemon grass oil and orange peel extract for 30 min i.e. T1, T2 and T3 were 3.99±0.04, 3.30±0.06 and 4.06±0.03 respectively. The mean values of the coliform count of control and T1 (lemon juice), T2 (lemon grass oil) and T3 (orange rind powder) differ significantly (p<0.05). The lowest coliform count was observed in T2 whereas the highest was observed in the untreated meat cutting board (control). This study showed log CFU/cm^2 reduction in the mean value of coliform count in T2 as compared with control (C). Anu (2015) [3] reported the occurance of more than 20 per cent *E coli* contamination from surface swabs collected from beef slaughter houses.

The mean value of Yeast and mould count of the meat cutting boards treated with lemon, lemon grass oil and orange peel extract for 30 min (T1, T2, and T3) with those of control(C) (Untreated meat cutting board) are presented in Table 2 and Figure 1. Yeast and moulds are important environmental contaminants which usually contaminate the carcass during the time of dehiding, evisceration, washing and retail sale. Considering the prevailing marketing practices of large animal carcasses in India, retailing of meat in open air and reuse of water will potentially increase carcass contamination with yeast and mould.

The mean value of yeast and moulds count (log cfu/cm^2) of untreated meat cutting board (control) sample was 2.58 ±.09. The mean yeast and mould count of meat cutting boards treated with lemon, lemon grass oil and orange peel extract for 30 min i.e. T1, T2 and T3 were 2.48±0.03, 2.16±0.04 and 2.54±0.05 respectively. The mean values of the yeast and mould count of control and treatment T2 differ significantly (p<0.05). The lowest yeast and moulds count was observed in T2 whereas the highest was observed in the untreated meat cutting board sample (control). As yeast and mould growth and meat spoilage are potentially damaging for the export meat industry, treating or washing decontaminating agents can be advocated. These results indicate that treating with lemon grass oil for 30 min will reduce the level of yeast and mould count in the meat cutting board surface.

Conclusion

Among all the three treatments, lemon grass oil (T2) resulted in significant reduction (p<0.05) in the mean values of TVC, coliform count and yeast and mould count when compared to control. Lemon grass oil can be used as effective alternative against hazardous chemical sanitizers used in meat industry without affecting the cost.

References

1. Adetunji VO, Isola TO. Antibiotic resistance of Escherichia coli, Listeria and Salmonella isolates from retail meat tables in Ibadan municipal abattoir, Nigeria. Afr. J Biotechnol. 2011; 10:5795-5799.
2. Al-Shuneigat J, SD Cox, JL Markham. Effects of a topical essential oil containing formulation on biofilm-forming coagulase-negative staphylococci. Lett. Appl. Microbiol. 2005; 41:52-55.
3. Anu PJ. Identification of critical control points in beef processing line with special reference to Enterohaemorrhagic *Escherichia coli*. *M.V.Sc thesis*, Kerala Veterinary and Animal Sciences University, Pookode, 2015, 86.
4. Beuchat LR, Cousine MA. Yeast and Molds. Compendium of methods for the microbiological examination of foods. (4th Ed.). APHA, Washington D.C, 2001, 209-213.
5. Burt SA. Essential oils: Their antibacterial properties and potential applications in foods: A review. Int. J Food Microbiol. 2004; 94:223-253.
6. Caccioni DRL, Guizzardi M. Inhibition of germination and growth of fruit and vegetable post-harvest pathogenic fungi by essential oil components. J Essential Oil Res. 1994; 6:173-179.
7. Çetin O, Kahraman T, Büyükünal SK. Microbiological evaluation of food contact surfaces at red meat processing plants in Istanbul, Turkey. Ital. J Anim. Sci. 2006; 5:277-283.
8. Emswiler BS, AW Kotula, DK Rough. Bactericidal effectiveness of three chlorine sources used in beef carcass washing. J Anim. Sci. 1976; 42:1445-1450.
9. Fisher K, Phillips CA. The effect of lemon, orange and bergamot essential oils and their components on the survival of Campylobacter jejuni, Escherichia coli O157, Listeria monocytogenes, Bacillus cereus and Staphylococcus aureus *in vitro* and in food systems. J. Appl. Microbiol. 2006; 101(6):1232-1240.
10. Frazier WF, Westhoff DC. Food Microbiology, Third edition. Tata McGraw-Hill Publishing Co. Limited, New-Delhi, India. 1978; l7-64:454-456.
11. Friedman M, Henika PR, Mandrell RE. Bactericidal activities of plant essential oils and some of their isolated constituents against Campylobacter jejuni Escherichia coli, Listeria monocytogenes, and Salmonella enterica. J food protection. 2002; 65(10):1545-1560.
12. Guarino C, Bartoli M, Izzi R, Calaprice A. Microbiological investigations on environmental pollution in slaughterhouses. Atti. Della Societa. Italiana Delle Sci. Vet. 1974; 27:684-687.
13. Hegazy AE, Ibrahium MI. Antioxidant activities of orange peel extracts. World Appl. Sci. J. 2012; 18(5):684-688.
14. Kelly CA, B Lynch, AJ McLoughlin. The effect of spray washing on the development of bacterial numbers and storage life of lamb carcasses. J Appl. Bacteriol. 1981; 57:335-359.
15. Lanciotti R, Gianotti A, Patrignani F, Belletti N, Guerzoni EM, Gardini F *et al*. Use of natural aroma compounds to improve shelf-life and safety of minimally processed fruits. Trends Food Sci. Tech. 2004; 15:201-208.
16. Lis-Balchin M, Deans SG. Bioactivity of selected plant essential oils against Listeria monocytogenes. J Appl. Bact. 1997; 82:759-762.
17. Miller AJ, Brown T, Call JE. Comparison of wooden and polyethylene cutting boards: potential for the attachment and removal of bacteria from ground beef. J Food Prot. 1996; 59:854-858.
18. Mishra AK, Dubey NK. Evaluation of some essential oils

for their toxicity against fungi causing deterioration of stored food commodities. Appl. Environ. Microbiol. 1994; 60:1101-1105.
19. Moleyar V, Narasimham P. Antibacterial activity of essential oil components. *Int*. J Food Microbiol. 1992; 16:337-342.
20. Morton RD. Total viable count. Compendium of methods for the microbiological examination of foods. (4th Ed.). APHA, Washington, D.C, 2001, 63-67.
21. Nesse LL, Sekse C, Berg K, Johannesen KC, Solheim H, Vestby LK *et al*. Potentially pathogenic Escherichia coli can form a biofilm under conditions relevant to the food production chain. Appl. Environ. Microbiol. 2014; 80:2042-2049.
22. Smulder FJ, Greer GG. Integrating microbial decontamination with organic acids in HACCP programmes for muscle foods: Prospects and controversies. Int. J Food Microbiol. 1998; 44(3):149-169.
23. Subba MS, TC Soumithri, R Suryanarayana Rao. Antimicrobial action of citrus oils. J Food Sci. 32:225-227.
24. Titus TC, JC Acton, L McCaskill, MG Johnson. Microbial persistence on inoculated beef plates sprayed with hypochlorite solutions. J Food Prot. 1978; 41:606-612.