Antibacterial activity of cinnamaldehyde and clove oil: effect on selected foodborne pathogens in model food systems and watermelon juice

S. Siddiqua · B. A. Anusha · L. S. Ashwini · P. S. Negi

Revised: 3 November 2014 / Accepted: 5 November 2014 / Published online: 14 November 2014
© Association of Food Scientists & Technologists (India) 2014

Abstract Natural additives for the control of microbial growth are in demand because consumers prefer them over synthetic ones. In the present investigation, the antibacterial activity of two natural preservatives, cinnamaldehyde and clove oil alone or in combinations was studied, and their potential as food preservative in model food systems and watermelon juice was evaluated. The cinnamaldehyde and clove essential oil showed minimum inhibitory concentration (MIC) at or below 5000 mg/l, and fractional inhibitory studies using both the oils showed synergistic effect. In artificially inoculated barley model food system and cabbage model food system, 2 MIC of oils was able to reduce the growth of the tested bacteria (more than 5 log) during 4 weeks storage at 37 °C, and similar reduction was also observed when combinations of oils were used at one eighth of MIC against *Bacillus cereus* and *Yersinia enterocolitica*, and one fourth of MIC against *Staphylococcus aureus* and *Escherichia coli*. Natural contaminants of watermelon juice were also reduced by the combination of one fourth of MIC of the oils, which was more effective than individual 2 MICs. These findings may be useful for food applications, but their effect on sensory quality of various foods need to be studied.

Keywords Clove oil · Cinnamaldehyde · Food systems · Fractional inhibitory concentration · Minimum inhibitory concentration

S. Siddiqua · B. A. Anusha · L. S. Ashwini · P. S. Negi (✉)
Fruit and Vegetable Technology Department, Council of Scientific and Industrial Research - Central Food Technological Research Institute, Mysore 570 020, India
e-mail: psnegi@yahoo.com

P. S. Negi
e-mail: psnegi@cftri.res.in

Introduction

Food safety is a fundamental concern for both consumers and the food industry, especially as the numbers of reported cases of food-associated infections continue to increase worldwide. Most plants produce antimicrobial secondary metabolites, either as part of their normal programme of growth and development or in response to pathogen attack or stress. These plant-based antimicrobials can be used as natural preservatives (Kumudavally et al. 2011; Das et al. 2012). Essential oils are naturally occurring terpenic mixtures isolated from various parts of plants by steam distillation or other methods. Essential oils are currently in demand, both in industry and academic research, and their antimicrobial properties against food spoilage microorganisms have been investigated in many studies (Burt 2004; Hernandez-Ochoa et al. 2011).

Cinnamon oil exerts strong antimicrobial properties against bacteria in in-vitro system (Tajkarimi et al. 2010; Das et al. 2012), and a few studies have reported its efficacy in food system alone (Andevary and Rezaei 2011); in combination with heat (Amalaradjou et al. 2010) or heat and acid (Binduheva and Negi 2014). The essential oil of clove (*Syzygium aromaticum*) also exhibits antimicrobial activity against the growth of bacteria and fungi, and significant antimicrobial effects of clove oil in in-vitro system (Fu et al. 2007) and in meat (Hernandez-Ochoa et al. 2011) was reported. However, the antimicrobial effectiveness of these essential oils in foods is not yet fully understood.

Essential oils show substantial activity when used in food systems but amounts required (33–100 times of in-vitro concentrations) are very high (Shelef et al. 1984), and such concentrations are generally much higher than the organoleptically acceptable levels. Higher concentrations of the essential oils are required probably due to the complex growth environment in food, which may provide protection to microbial cells (Gill et al. 2002; Gutierrez et al. 2009; Smith-Palmer

et al. 2001). It has been shown that combining different antimicrobial substances can lead to a broad spectrum of activity which can increase their effectiveness in foods (Ghraiiri and Hani 2013; Goni et al. 2009; Gutierrez et al. 2008). Therefore, in the present study, attempts have been made to study the antibacterial activity of cinnamaldehyde and clove oil alone or in combinations in in-vitro conditions, and to investigate their potential as food preservative in barley model food system and cabbage model food system, and in watermelon juice.

Materials and methods

Essential oils

The cinnamaldehyde and clove oil were purchased from Loba Chemie Pvt. Ltd. (Mumbai, India). The cinnamaldehyde supplied by the company is a clear yellow coloured liquid having strong odour of cinnamon with a density of 1.045 g/ml and 99.75 % purity. The clove oil is a yellow coloured clear liquid having eugenol content of 85.08 % (v/v) with a density of 1.058 g/ml and refractive index of 1.530. The oil (1 ml) was dissolved in distilled water (total volume 10 ml) using 100 µl of tween 80 (SRL, Mumbai, India) for use in antibacterial studies.

Antibacterial activity assay in-vitro

The bacterial cultures used in present study were *Bacillus cereus* (F 4810, Public Health Laboratory, London, UK), *Staphylococcus aureus* (FRI 722, Public Health Laboratory, The Netherlands), *Escherichia coli* (MTCC 108, Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India) and *Yersinia enterocolitica* (MTCC 859, Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India). The cinnamaldehyde and clove oil were tested against selected bacteria by agar dilution method (Negi et al. 1999). In brief, different concentration of cinnamaldehyde and clove oil were added individually in to the flasks containing 20 ml of molten nutrient agar media (~50 °C), and 100 µl of bacterium to be tested (10^3 cfu/ml) was inoculated. The contents of the flask were poured into the sterilized petriplates under aseptic conditions, and the plates were incubated for 24 h at 37 °C and observed for bacterial growth. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the compound capable of inhibiting the complete growth of bacterium in question.

Determination of fractional inhibitory concentration (FIC)

The checkerboard method was performed to obtain the FIC index of clove oil and cinnamaldehyde combinations against

the test bacterium in the nutrient agar media (Schelz et al. 2006). To the flasks containing 20 ml of molten nutrient agar media (~50 °C), a combination of different concentration of cinnamaldehyde and clove oils diluted two fold (MIC/4, MIC/8) were added, and 100 µl of each bacterium (10^3 cfu/ml) to be tested was inoculated before pouring the contents into the sterilized petriplates. The plates were incubated for 24 h at 37 °C and observed for bacterial growth. The MIC for each combination was defined as the lowest concentration of the combination capable of inhibiting the complete growth of bacterium in question.

The FIC indices were calculated as $FIC_{\text{clove}} + FIC_{\text{cinnamaldehyde}}$, where $FIC_{\text{clove}} = (\text{MIC}_{\text{clove combination}} / \text{MIC}_{\text{clove alone}})$ and $FIC_{\text{cinnamaldehyde}} = (\text{MIC}_{\text{cinnamaldehyde combination}} / \text{MIC}_{\text{cinnamaldehyde alone}})$. The results were interpreted as synergy ($FIC < 0.5$), addition ($0.5 < FIC < 1$), indifference ($1 < FIC < 4$) or antagonism ($FIC > 4$).

Antibacterial activity assay in food systems

Barley soup was prepared by adding barley powder (obtained by grinding barley seeds) to the distilled water (10 %, w/v), and cabbage suspension by grinding cabbage along with sterile deionised water (50 % w/v) in a mixer followed by filtration using muslin cloth (Catherine et al. 2012). The barley soup and cabbage suspension were dispensed individually in portions of 50 ml into 250 ml conical flask and sterilized (121 °C for 15 min). To the flasks containing barley soup or cabbage suspension, the essential oils (cinnamaldehyde and clove oil) were added in different concentrations (MIC and 2MIC alone, and combination of MIC/4 of each oil and MIC/8 of each oil), and it was inoculated with approximately 10^5 cells of each bacterium separately. Flasks with tween 80 in water (100 µl in 10 ml, without added essential oils) served as control. The flasks were then incubated at 37 °C for 28 days. The barley soup or cabbage suspension was withdrawn at regular interval to check the level of contamination by pour plate method using plate count agar in triplicates. The colonies grown after 24 h of incubation at 37 °C were counted, and count was expressed as log cfu/ml.

Antibacterial activity against natural contaminants of watermelon juice

The watermelon juice was purchased from the local vendor and transferred to laboratory within an hour. The juice (50 ml) was dispensed in sterilized conical flasks (250 ml) under aseptic conditions. MIC and 2 MIC of the oils individually, and combination of one-fourth MIC of each oil and combination of one-eighth MIC of each oil were added to the flasks containing juice. The juice with tween 80 in water (100 µl in 10 ml, without added essential oils) was used as control. The total aerobic bacterial count of treated and untreated juice was

estimated at regular interval using plate count agar during its storage at 37 °C for 7 days. The colonies grown after 24 h of incubation at 37 °C were counted. The plating was done in triplicate and the count was expressed as log cfu/ml. The reproducibility of the experiment in terms of the background microflora of the fresh juice was ascertained by terminating the experiments showing unusually high or low count in control at 0 day.

Statistical analysis

The data on MIC and FIC were same for all replicates, therefore were represented as such. The surviving bacteria in different food models and watermelon juice after treatment with various essential oil concentrations were represented as mean±SD. The effect of essential oil treatment was analyzed by one way ANOVA and Turkey Kramer Multiple Comparison Test was used to compare statistical differences ($p<0.05$) among various treatments and concentrations for a bacterium in a model system at the end of each storage period.

Results and discussion

Antibacterial activity of essential oils in-vitro

Growth inhibition studies were conducted in vitro to investigate the antimicrobial action of the cinnamaldehyde and clove oil against the tested microorganisms. The cinnamaldehyde and clove oil were able to reduce the bacterial population completely at or below 5000 mg/l level for all the bacteria tested in present investigation (Table 1). The growth inhibition pattern showed that clove oil was more effective than cinnamaldehyde against tested Gram negative bacteria as it inhibited the growth of *E. coli* and *Y. enterocolitica* completely at 4500 mg/l (MIC of 5000 mg/l for cinnamaldehyde), whereas cinnamaldehyde was more effective against tested Gram-positive bacteria as compare to clove oil, and it inhibited the complete growth of *S. aureus* at 1875 mg/l and

Table 1 MIC of cinnamon and clove oils against different foodborne pathogens

	Clove oil (mg/l)	Cinnamaldehyde (mg/l)
<i>Escherichia coli</i>	4500	5000
<i>Staphylococcus aureus</i>	4500	1875
<i>Bacillus cereus</i>	2750	2000
<i>Yersinia enterocolitica</i>	4500	5000

Result of four replication (concentration at which no colony was formed after 24 h incubation)

that of *B. cereus* at 2000 mg/l. Although, higher activity of cinnamaldehyde and clove in vapour phase using zone inhibition method is reported (Goni et al. 2009), the MIC values for clove oil against *S. epidermidis*, *E. coli* and *Candida albicans* (6000–50,000 mg/l) using broth micro-dilution method (Fu et al. 2007) were higher than the values observed in the present study. The antimicrobial activity of essential oils might vary considerably depending on number of factors like the botanical source of the plant, time of harvesting, stage of development, and method of extraction, which can significantly affect the active constituents of the essential oils. Also the bacterial strains being tested and bacterial load has effect on antibacterial activity of the essential oils (Singh et al. 2003). In general, the cinnamaldehyde and clove oil showed higher antimicrobial activity (lower MIC values) against Gram-positive than against Gram-negative bacteria tested in the present study, a fact previously observed with essential oils (Nostro et al. 2000) as well as with several plant extracts (Negi 2012).

FIC values

The checkerboard method was performed to obtain the FIC index of clove oil and cinnamaldehyde combinations. Various combinations of essential oils showed synergistic FIC values (FIC<0.5) against tested organisms at their one-fourth to one-eighth MIC values (Table 2). The other combinations of oil greater than MIC/4 and MIC/8 revealed an indifferent interaction, and the FIC indices varied from 1.0 to 2.0 (data not shown). Various effects of combining antimicrobial compounds ranging from synergistic to indifference are reported in literature. An increase in antimicrobial efficacy of essential oils was observed when they were used in combination

Table 2 Combinations showing synergistic FIC values for cinnamon and clove oils against different foodborne pathogens

	Clove oil	Cinnamaldehyde	FIC value
<i>E. coli</i>	MIC/8	MIC/4	0.50
	MIC/4	MIC/4	0.25
	MIC/4	MIC/8	0.38
<i>Y. enterocolitica</i>	MIC/8	MIC/8	0.25
	MIC/4	MIC/4	0.50
	MIC/4	MIC/8	0.38
<i>B. cereus</i>	MIC/4	MIC/8	0.37
	MIC/8	MIC/8	0.25
	MIC/8	MIC/4	0.38
<i>S. aureus</i>	MIC/4	MIC/8	0.38
	MIC/8	MIC/8	0.38
	MIC/4	MIC/4	0.25

Result of four replication

Fig. 1 a The antibacterial effect of essential oils alone or in combination in barley model food system. b The antibacterial effect of essential oils alone or in combination in cabbage model food system

Fig. 1 (continued)

Fig. 2 The antibacterial effect of essential oils alone or in combination against natural contaminants in watermelon juice

(Shelef et al. 1984; Gutierrez et al. 2008, 2009). Combination of thyme essential oil and enterocin A showed synergistic antibacterial effect on *Listeria monocytogenes* and *E. coli* (Ghraihi and Hani 2013), however, the combinations of *Cymbopogon giganteus* and *C. citratus* essential oils exerted synergistic, additive and indifferent antimicrobial effects depending on the microorganism and used concentration (Bassole et al. 2011).

Antibacterial effect of essential oils in model food systems

The antibacterial effectiveness of clove oil, cinnamaldehyde and their combinations were tested against *E. coli*, *S. aureus*, *B. cereus* and *Y. enterocolitica* by artificially inoculating them in barley (Fig. 1a) and cabbage model food systems (Fig. 1b). In both the food systems, there was a marked increase in bacterial population in control samples, whereas in MIC, the counts decreased as the incubation period was increased. Survival of only a few bacterial cells was seen in 2MIC treatment throughout the incubation period in both the food model systems. In cabbage food system, MIC treatments showed reduced growth, and 2 MICs resulted in lower viable counts as compare to controls. Almost similar trend for inhibition of growth was observed in barley food system also. Although, the count for most of the treatments in both the systems against all the bacteria was statistically similar ($p < 0.05$) to each other on the day of treatment, the treatments showed significantly lower count throughout storage (except most of the combinations after 21 days storage in case of barley model system for *B. cereus* and *S. aureus*, cabbage model system for *E. coli*, cabbage and barley model systems for *Y. enterocolitica*; and after 7 days storage for *E. coli* in case of barley model system) as compare to control. Use of

combination of essential oils was effective in controlling the growth of bacteria in both the food systems in the present study, albeit at much lower concentrations (MIC/ 8 of each oil for *B. cereus* and *Y. enterocolitica*; and MIC/ 4 of each oil for *E. coli* and *S. aureus*), which also showed synergistic effect in in-vitro condition (Table 2). At the end of storage, combined MIC/8 treatment showed significantly lower count than individual 2 MICs against *B. cereus* in cabbage model system. Similarly, at the end of 14 days storage, combined MIC/4 treatment showed significantly lower count than individual 2 MICs against *S. aureus* in barley model system. However, MIC/4 and MIC/8 had statistically similar ($p < 0.05$) effect on *S. aureus*, *E. coli* and *Y. enterocolitica* in both food model systems, and *B. cereus* in cabbage model system at the end of 14, 21 and 28 days storage. We also observed increase in microbial load in treated samples towards the end of storage period in few treatments such as MIC and 2 MICs of cinnamaldehyde against *S. aureus* and *Y. enterocolitica*, and 2 MIC of clove oil against *B. cereus* in cabbage model system; and MIC, 2 MICs and combined MIC/4 treatment against *B. cereus*, and combined MIC/4 and MIC/8 treatment against *Y. enterocolitica* in barley model system (Fig. 1 a and b).

In both the food systems, the decrease in bacterial population with addition of essential oils was concentration dependent, and the inhibitory activity of the essential oils was lower in food systems as compared to in-vitro system. Differences in inhibitory activity of essential oils in different food model systems have been reported in literature (Gutierrez et al. 2009). Clove oil did not have any significant effect on *E. coli* O157: H7 count in cooked beef at 3 MIC level as compare to control, however it showed better inhibitory effect in blanched spinach at similar concentration (Moreira et al. 2007). Similarly, a fourfold decrease in antimicrobial activity (as compare

to in-vitro activity) of *Litsea cubeba* essential oil against *Vibrio parahaemolyticus*, *L. monocytogenes* and *Lactobacillus plantarum* was observed in tofu, oysters and orange-milk beverage, respectively (Wei et al. 2011). Although synergistic effect of some oil combinations against several bacteria has been reported under in-vitro conditions (Gutierrez et al. 2008), no synergistic effect of oregano and cranberry essential oil combination was observed against *L. monocytogenes* in fish and meat system (Liu and Yang 2012).

Antibacterial effect of essential oils in watermelon juice

The control of natural bacterial contaminants present in watermelon juice by both the essential oils and their combinations was analyzed, and it was observed that all the treated juices had significantly ($p < 0.05$) lower counts as compare to control juice on all days of analysis. Although the level of contaminant did not show a definite trend for all the treatments on all days of analysis, clove oil at 2 MIC was the most effective ($p < 0.05$) in controlling bacterial growth initially and at the end of day 5 and 7, and combination of each oil at one eighth MIC level was significantly ($p < 0.05$) least effective (Fig. 2). Cinnamaldehyde at 2 MIC level showed least contaminants at day 1 (statistically similar to 2 MIC of clove), but combination of one fourth MIC of each oil showed significantly higher ($p < 0.05$) inhibition of bacterial growth in watermelon juice than their individual 2 MIC levels at day 5, which was also more effective ($p < 0.05$) than all the treatments and as effective ($p < 0.05$) as 2 MIC of clove oil after day 7. Although variability in background microflora of the fresh juice was minimized by terminating the experiments showing unusually high or low count in control at 0 day, the composition of the natural contaminants of watermelon juice may affect the antibacterial activity of the oils and their combinations. Application of the *Origanum vulgare* and *Rosmarinus officinalis* essential oils alone (MIC level) or in mixture (one fourth MIC each) is reported to significantly reduce the number of *L. monocytogenes*, *Y. enterocolitica*, *Aeromonas hydrophila* and *Pseudomonas fluorescens* in vegetable broth and in experimentally inoculated fresh-cut vegetables (De Rapper et al. 2012), and in our study also, the natural contaminants of watermelon juice were effectively controlled by both the essential oils and their combinations.

Conclusions

This study showed that the essential oils of cinnamaldehyde and clove oils possess antibacterial activity and their combinations were effective in inhibiting the growth of artificially inoculated foodborne pathogens in model food system as well as natural contaminants of watermelon juice. Better

antibacterial activity of combinations of oils than individual oil observed in studied food systems here has industrial relevance as it will help in better preservation of foods due to broader spectrum of antibacterial activity. The combinations of oils will also be helpful in reducing concentration of individual oil, thereby minimizing the undesirable impact on sensory properties, but their efficacy in complex foods, and toxicity and safety needs further study.

References

- Amalaradjou MAR, Baskaran SA, Ramanathan R, Johny AK, Charles AS et al (2010) Enhancing the thermal destruction of *Escherichia coli* O157:H7 in ground beef patties by trans-cinnamaldehyde. Food Microbiol 27:841–844
- Andevarti GT, Rezaei M (2011) Effect of gelatin coating incorporated with cinnamon oil on the quality of fresh rainbow trout in cold storage. Int J Food Sci Technol 46:2305–2311
- Bassole IHN, Lamien-Meda A, Bayala B, Obame LC, Ilboudo AJ et al (2011) Chemical composition and antimicrobial activity of *Cymbopogon citratus* and *Cymbopogon giganteus* essential oils alone and in combination. Phytomedicine 18:1070–1074
- Binduheva U, Negi PS (2014) Efficacy of cinnamon oil to prolong the shelf-life of pasteurised acidified and ambient stored papaya pulp. Acta Aliment 43:378–386
- Burt S (2004) Essential oils: their antibacterial properties and potential application in foods—a review. Int J Food Microbiol 94:223–253
- Catherine AA, Deepika H, Negi PS (2012) Antibacterial activity of eugenol and peppermint oil in model food systems. J Essent Oil Res 24:481–486
- Das M, Rath CC, Mohapatra UB (2012) Bacteriology of a most popular street food (*Panipuri*) and inhibitory effect of essential oils on bacterial growth. J Food Sci Technol 49:564–571
- De Rapper S, Van-Vuuren SF, Kamatou GPP, Viljoen AM, Dagne E (2012) The additive and synergistic antimicrobial effects of select frankincense and myrrh oils—a combination from the pharaonic pharmacopoeia. Lett Appl Microbiol 54:352–358
- Fu YJ, Zu YG, Chen LY, Shi XG, Wang Z et al (2007) Antimicrobial activity of clove and rosemary essential oils alone and in combination. Phytother Res 21:989–994
- Ghraiiri T, Hani K (2013) Enhanced bactericidal effect of enterocin A in combination with thyme essential oils against *L. monocytogenes* and *E. coli* O157:H7. J Food Sci Technol. doi:10.1007/s13197-013-1214-5
- Gill AO, Delaquis P, Russo P, Holley RA (2002) Evaluation of antilisterial action of cilantro oil on vacuum packed ham. Int J Food Microbiol 73:83–92
- Goni P, Lopez P, Sanchez C, Gomez-Lus R, Becerril R, Nerin C (2009) Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. Food Chem 116:982–989
- Gutierrez J, Barry-Ryan C, Bourke P (2008) The antimicrobial efficacy of plant essential oil combinations and interaction with food ingredients. Int J Food Microbiol 124:91–97
- Gutierrez J, Barry-Ryan C, Bourke P (2009) Antimicrobial activity of plant essential oils using food model media, efficacy, synergistic potential and interactions with food components. Food Microbiol 26:142–150
- Hernandez-Ochoa L, Aguirre-Prieto YB, Nevarez-Moorillon GV, Gutierrez-Mendez N, Salas-Munoz E (2011) Use of essential oils

- and extracts from spices in meat protection. J Food Sci Technol. doi: [10.1007/s13197-011-0598-3](https://doi.org/10.1007/s13197-011-0598-3)
- Kumudavally KV, Tabassum A, Radhakrishna K, Bawa AS (2011) Effect of ethanolic extract of clove on the keeping quality of fresh mutton during storage at ambient temperature (25 ± 2 °C). J Food Sci Technol 48:466–471
- Liu TT, Yang TS (2012) Antimicrobial impact of the components of essential oil of *Litsea cubeba* from Taiwan and antimicrobial activity of the oil in food system. Int J Food Microbiol 156:68–75
- Moreira MR, Ponce AG, Del Valle CE, Roura SI (2007) Effects of clove and tea tree oils on *Escherichia coli* O157:H7 in blanched spinach and minced cooked beef. J Food Process Preserv 31:379–391
- Negi PS (2012) Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application. Int J Food Microbiol 156:7–17
- Negi PS, Jayaprakasha GK, Rao LJM, Sakariah KK (1999) Antibacterial activity of turmeric oil—a byproduct from curcumin manufacture. J Agric Food Chem 47:4297–4300
- Nostro A, Germano MP, D'angelo V, Marino A, Cannatelli MA (2000) Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol 30:379–384
- Scholz Z, Molnar J, Hohmann J (2006) Antimicrobial and antiplasmid activities of essential oils. Fitoterapia 77:279–285
- Shelef LA, Jyothi EK, Bulgarelli MA (1984) Growth of enteropathogenic and spoilage bacteria in sage-containing broth and foods. J Food Sci 49:737–740
- Singh A, Singh RK, Bhunia AK, Singh N (2003) Efficacy of plant essential oils as antimicrobial agents against *Listeria monocytogenes* in hotdogs. LWT—Food Sci Technol 36:787–794
- Smith-Palmer A, Stewart J, Fyfe L (2001) The potential application of plant essential oils as natural food preservatives in soft cheese. Food Microbiol 18:463–470
- Tajkarimi MM, Ibrahim SA, Cliver DO (2010) Antimicrobial herb and spice compounds in food. Food Control 21:1199–1218
- Wei QY, Xiong JJ, Jiang H, Zhang C, Ye W (2011) The antimicrobial activities of the cinnamaldehyde adducts with amino acids. Int J Food Microbiol 150:164–170