Geraniol and Linalool Loaded Nanoemulsions and Their Antimicrobial Activity

Article · November 2017

DOI: 10.15835/buasvmcn-asb:0025

7 authors, including:

Igori Balta
University of Agricultural Sciences and Veterinary Medicine
6 PUBLICATIONS **6** CITATIONS
SEE PROFILE

Lucian Brinzan
1 PUBLICATION **5** CITATIONS
SEE PROFILE

Alexandros Stratakos
Queen's University Belfast
51 PUBLICATIONS **428** CITATIONS
SEE PROFILE

Mark Linton
Agri-Food and Biosciences Institute
51 PUBLICATIONS **1,200** CITATIONS
SEE PROFILE

Geraniol and Linalool Loaded Nanoemulsions and Their Antimicrobial Activity

Igori BALTA[1ǂ], Lucian BRINZAN[1ǂ], Alexandros Ch. STRATAKOS[2], Mark LINTON[2], Carmel KELLY[2], Laurette PINKERTON[2], Nicolae CORCIONIVOSCHI[2]*

[1] University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Faculty of Animal Science and Biotechnologies. Cluj-Napoca, Romania
[2]Agri-Food and Biosciences Institute, Bacteriology Branch, Belfast, United Kingdom.
*Corresponding author, e-mail: Nicolae.Corcionivoschi@afbini.gov.uk
ǂEqual contribution

Bulletin UASVM Animal Science and Biotechnologies 74(2)/ 2017
Print ISSN 1843-5262; Electronic ISSN 1843-536X
DOI:10.15835/buasvmcn-asb: 0025

ABSTRACT

Geraniol and linalool have been found to be effective against foodborne microorganisms *in vitro*. However, due to their hydrophobic nature, it is difficult to achieve an even dispersion in foods with high water content resulting in dramatic loss of activity. The aim of the study was to fabricate geraniol or linalool nanoemulsions and investigate their effect against *Escherichia coli* K12, *Listeria innocua* and *Pseudomonas lundensis* in a meat simulation medium. The agar diffusion assay revealed that both geraniol and linalool had a potent antimicrobial activity against all bacteria. Dynamic light scattering showed that geraniol and linalool nanoemulsions had a mean diameter of 68.22±2.46 and 173.59±4.15 nm, respectively. Killing assay results showed that both nanoemulsions were able to significantly reduce *E. coli* and *L. innocua* counts by approx. 3 log CFU/ml. *Ps. lundensis* proved to be more resistant to both nanoemulsions showing a reduction of approx. 1.2 log CFU/ml,. Overall, this study showed that nanoemulsions loaded with geraniol or linalool represent a promising antimicrobial system to improve food preservation and food safety.

Keywords: *antimicrobial, geraniol, linalool, nanoemulsion*

INTRODUCTION

Foodborne contamination has posed a major public health concern worldwide for a very long time (Settanni *et al.*, 2012). In the U.S.A., the number of foodborne illnesses is estimated to about 5 million reported cases that lead towards 60,000 hospitalizations and 1800 deaths and about half of foodborne outbreaks are linked to the consumption of contaminated meat (Magnus M., 2008). Meat and meat products are prone to microbial spoilage during slaughtering, processing and storage because they possess an ideal nutrient matrix that can favour the growth of pathogenic and spoilage microorganisms. Essential oils have been gaining importance as food preservatives as many studies have found that they possess significant antimicrobial properties against a broad range of foodborne pathogens (Zhang *et al.*, 2016). These substances are considered "natural" and their use for commercial applications is very attractive since many consumers are now concerned about the addition of synthetic compounds to foods (Burt, 2004). Geraniol, the main component of rose oil, palmarosa oil, and citronella oil and linalool the main compounds of coriander essential oil have been found to have antimicrobial activity *in vitro* (Burt, 2004). However, due to their hydrophobic nature, it is difficult to achieve an even dispersion in foods with high water content which results in dramatic loss of activity. Different antimicrobial delivery systems have been proposed to improve effectiveness of antimicrobials in food matrices by increasing their dispersion and protecting them from coming in to contact with food components (e.g. proteins, lipids) and losing their activity (Xiao *et al.*, 2011; Zahi *et al.*, 2015). A potential

Tab 1. Antibacterial activity of pure geraniol and linalool against *E. coli* K12, *L. innocua* and *P. lundensis*. Values represent the diameter of inhibition zone in mm ± standard deviation.

Bacteria	*E.coli* K12	*Ps. lundensis*	*L. innocua*
Geraniol	11.78 ± 0.55 mm	12.50 ± 0.93 mm	13.83 ± 0.35 mm
Linalool	18.32 ± 1.17 mm	14.55 ± 1.14 mm	16.50 ± 2.11 mm

alternative to current recommended methods is the use of emulsified essential oils (Landry *et al.*, 2014). This method has recently been reported to be suitable for application in the food industry for fabricating effective antimicrobial nanoemulsions from essential oils (Chang *et al.*, 2013). Based on these findings and the fact that geraniol and linalool are good antimicrobial agents, the emulsification method was used to produce nanoemulsions. The purpose of this study was to characterise the geraniol and linalool nanoemulsions produced and investigate their effectiveness against *Escherichia coli* K12, *Listeria innocua*, *Pseudomonas lundensis* in a meat simulation medium at different time intervals.

MATERIALS AND METHODS

Cultures

Escherichia coli K12, *Listeria innocua* and *Pseudomonas lundensis* were cultured for 24h in brain-heart infusion broth at 37, 37 and 30°C, respectively.

Disc diffusion assay

Antimicrobial activity was evaluated by the disc diffusion method according by measuring the diameter of the inhibition zone around the disc (6 mm) for each of the different antimicrobial agents.

Formation of antimicrobial nanoemulsions

The preparation of the antimicrobial nanoemulsion was based on a method previously reported by Chang *et al.*, (2013). Geraniol or Linalool (4 g) (Sigma–Aldrich) was added to 6 g of medium chain triglyceride (MCT) oil (Miglyol 812, Witten, Germany) and thoroughly mixed for 5 min at 125 RPM. Once mixed, Tween 80® (10 g) (Sigma–Aldrich) was added to the oil mixture and mixed for another 5 min at 125 RPM. The oil/Tween 80 mixture (20 g) was titrated, at a rate of 2 mL/min, into 80 g of 5.0 mM sodium citrate buffer (pH 3.4) containing a magnetic stirring bar set to 600 RPM and allowed to mix for an additional 15 min. The emulsion was filter sterilized through a sterile 0.22 µm filter and stored in sterile 50 mL tubes at 2–5 °C for up to 1 week.

Characterisation of nanoemulsions

Droplet size was measured using dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments, UK). This instrument determines the particle size from intensity–time fluctuations of a laser beam (633 nm) scattered from a sample at an angle of 173°.

Killing assay

Nanoemulsions at a geraniol and linalool concentration of 4000 ppm were prepared in sterilized meat simulation medium (20 g of bacteriological peptone, 16 g of Lab Lemco, 8 g of yeast extract, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.038 g of $MnSO_4 \cdot H_2O$, 1 ml of Tween 80, 5 g of lactic acid (sterilized separately), 40 g of NaCl. Overnight *E. coli* K12, *L. innocua* and *Ps. lundensis* cultures in BHI broth were washed once in PBS and inoculated at 5 log CFU/ml to the corresponding nanoemulsion solutions, mixed and incubated at 37, 37 and 30^0C statically for 0, 4h, and 24h. The survival of all bacteria at a specific nanoemulsion and incubation time was enumerated by serial dilution and plating onto TSAYE agar. Control treatment did not contain any nanoemulsion.

Statistical analysis

The experiment was performed three times on different occasions in order to obtain three independent replicates. The data are presented as the mean ± SD. Differences between means were determined by t-test and ANOVA. The Tukey's test was used to compare differences amongst means. Differences were defined as significant at $p < 0.05$.

RESULTS AND DISCUSSION

Antimicrobial activity of pure geraniol and linalool as assessed with the disc diffusion method

In the present study, the diameter of the inhibition zone was used to evaluate the antimicrobial efficiency of geraniol and linalool against

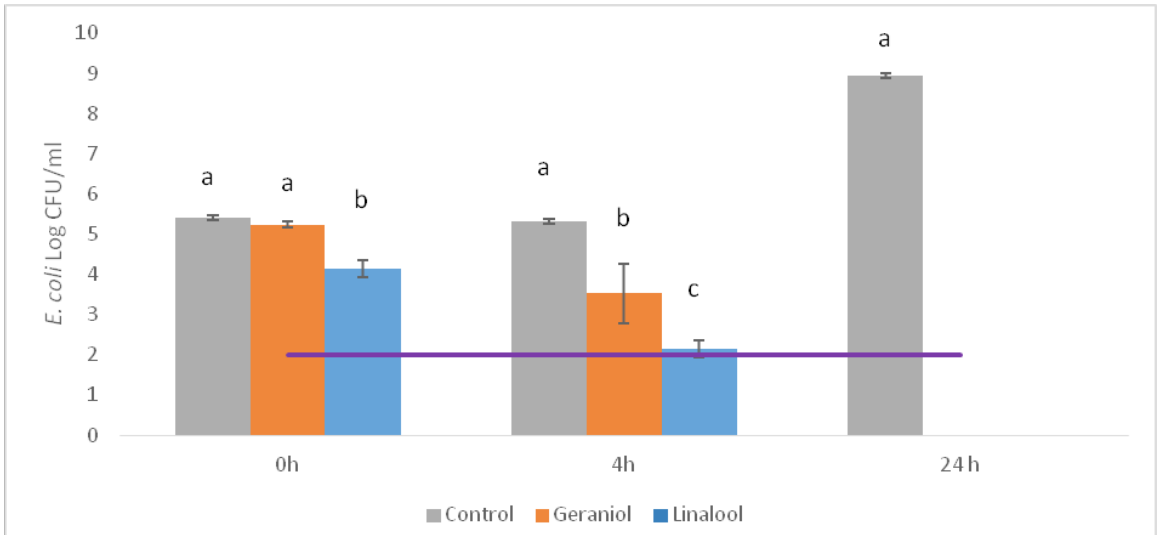

Fig. 1. Effect of geraniol and linalool nanoemulsions at a concentration of 4000 ppm on *E. coli* counts in meat simulation medium at different time intervals. Each point represents the mean ± standard deviation. Different letters for each time point denote statistically significant differences ($p < 0.05$). The line represents the detection limit. *E. coli* counts for geraniol and linalool nanoemulsions were below the detection limit.

E. coli K12, *L. innocua* and *Ps. lundensis*. Results in Table 1 show that both antimicrobials had a potent antibacterial effect against all bacteria tested. Linalool was most effective against *E. coli* (18.32 ± 1.17 mm) whereas geraniol was most effective against *L. innocua* (13.83 ± 0.35 mm). Similar antimicrobial activity has been found for carvacrol against O157 and non-O157 *E. coli* (Stratakos et al. 2017).

Characterisation of nanoemulsions

Results showed that both geraniol and linalool could be encapsulated in nanoemulsions. Characterisation with dynamic light scattering showed that the geraniol and linalool nanoemulsions had a mean diameter of 68.22 ± 2.46 and 173.59 ±4.15 nm, respectively. The polydispersity index for geraniol and linalool was also determined and was found to be 0.20 ± 0.005 and 0.24 ± 0.006, respectively. Landry *et al.* (2014) found similar results when they prepared nanoemulsions loaded with carvacrol with a mean droplet diameters of approx. 100 nm, as determined by dynamic light scattering.

Effect of geraniol and linalool nanoemulsions on the survival of *E. coli* in meat simulation medium.

Results presented in Figure 1 show that both nanoemulsions were effective against *E. coli* K12 in the meat simulation medium. The linalool nanoemulsion resulted in a significant reduction in *E. coli* counts immediately after treatment (approx. 1 log CFU/ml). At 4 h incubation the linalool nanoemulsion reduced counts by 3.17 log whereas the geraniol nanoemulsion by 1.8 log CFU ml. Both nanoemulsions were able to reduce *E. coli* counts below the detection limit which corresponds to >3 log reduction in *E. coli* counts, compared to the initial inoculum (approx. 5 log CFU/ml), after 24h incubation.

According to Barbosa *et al.*, (2009) oregano, thyme, basil, marjoram, lemongrass, ginger, and clove essential oils showed a significant reduction against *S. aureus*, *L. monocytogenes*, *E. coli*, and *Salmonella* Enteritidis *in vitro*. However, when these pathogens were artificially inoculated on irradiated beef meat resulted in a non-statistically significant reduction in the pathogen counts (<1 log CFU/g). Revealing loss of their activity when used in real food systems.

Effect of geraniol and linalool nanoemulsions on the survival of *L. innocua* in meat simulation medium.

Results presented in Figure 2 show, in this case as well, that both nanoemulsions were effective against *L. innocua* in the meat simulation medium. Untreated samples (control) grew rapidly, as expected. However, in this case both nanoemulions proved to be equally effective with *L. innocua* counts not differing significantly ($p > 0.05$) throughout the incubation. At 24h both

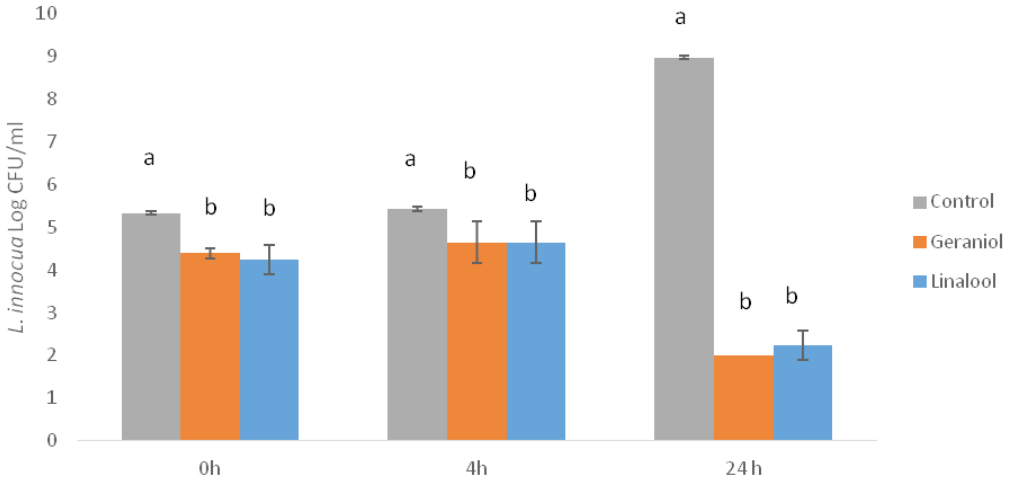

Fig. 2. Effect of geraniol and linalool nanoemulsions at a concentration of 4000 ppm on *L. innocua* counts in meat simulation medium at different time intervals. Each point represents the mean ± standard deviation. Different letters for each time point denote statistically significant differences ($p < 0.05$).

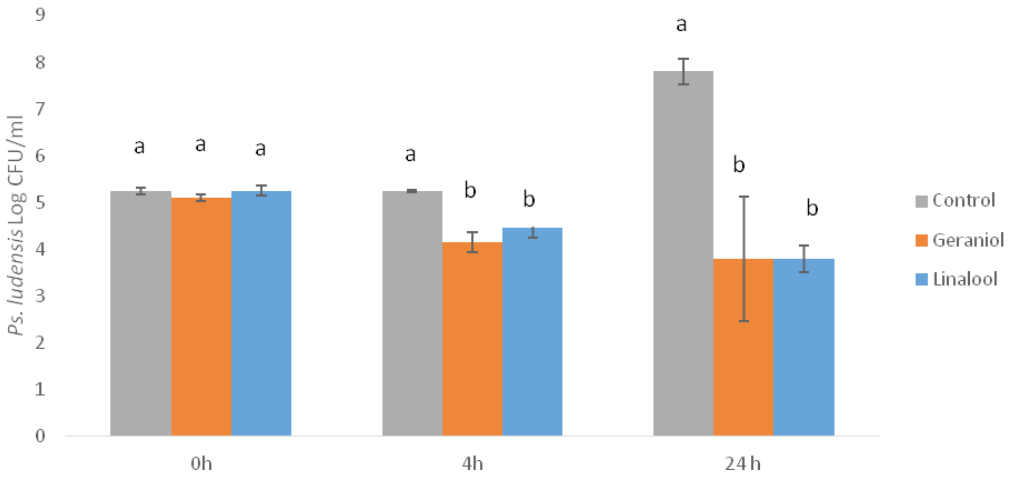

Fig. 3. Effect of geraniol and linalool nanoemulsions at a concentration of 4000 ppm on *Ps. lundensis* counts in meat simulation medium at different time intervals. Each point represents the mean ± standard deviation. Different letters for each time point denote statistically significant differences ($p < 0.05$).

nanoemulsions reduced counts by approx. 3 logs, compared to the initial inoculum (approx. 5 log CFU/ml).

Similar results were found in the study of Maté *et al.*, (2016) in which the antibacterial effect of nanoemulsions loaded with the natural compound d-limonene against *Listeria monocytogenes* in tryptic soy broth growth medium, chicken broth, and vegetable cream, was investigated. The counts of *L. monocytogenes* decreased approx. 3 log cycles in all growth media tryptic soy broth growth medium, chicken broth, and vegetable cream. The study also showed greater effectiveness when applying D-limonene in form of nanoemulsion than when applying it directly,

Effect of geraniol and linalool nanoemulsions on the survival of *Ps. lundensis* in meat simulation medium.

Ps. lundensis is one of the most prominent species of *Pseudomonas* that cause spoilage of meat during storage. Figure 3 presents the effect of geraniol and linalool nanoemulsions against this spoilage microorganism. In this case, there was no immediate effect on *Ps. Lundensis* with the counts of all three treatments not differing significantly ($p > 0.05$). After 4h incubation the geraniol and linalool nanoemulsion treatments were significantly lo-

wer ($p < 0.05$) compared to the control (4.15 and 4.45 log CFU/ml, respectively). After 24h, *Ps. Lundensis* counts were further reduced to 3.79 and 3.80 log CFU/ml for the geraniol and linalool nanoemulsions, respectively.

In summary, two food-grade nanoemulsions loaded with geraniol or linalool were tested for their efficacy against *E. coli* K12, *L. innocua* and *Ps. lundensis* in a meat simulation system. Both of the nanoemulsions fabricated and tested in this study showed very promising results. Geraniol and linalool nanoemulsions were able to significantly reduce *E. coli* and *L. innocua* counts. Also, although both nanoemulsions were able to significantly reduce *Ps. Lundensis*, this bacterium appeared to be less susceptible to the action of the nanoemulsions compared to *E. coli* K12 and *L. innocua*. Nanoemulsion technology could potentially help resolve issues of solubility and stability of hydrophobic antimicrobials in the food industry. With greater understanding of the system as a whole, they may find a broad range of applications in food preservation and food safety. Therefore, it would be useful in future studies to test the efficacy of the antimicrobial nanoemulsions developed here in real food systems to establish their range of efficacy.

REFERENCES

1. Barbosa LN, Rall VLM, Fernandes AAH, Ushimaru PI, da Silva Probst I, Fernandes JrA (2009). Essential oils against foodborne pathogens and spoilage bacteria in minced meat. Foodborne pathogens and disease, 6, 725-728.
2. Burt S (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. International journal of food microbiology, 94, 223-253.
3. Manya M (2008). Essentials of Infectious Disease Epidemiology. Essential Public Health. United States-ISBN-10:0763738786.
4. Chang Y, McLandsborough L, McClements DJ (2013). Physicochemical properties and antimicrobial efficacy of carvacrol nanoemulsions formed by spontaneous emulsification. Journal of agricultural and food chemistry, 61, 8906-8913.
5. Landry KS, Chang Y, McClements DJ, McLandsborough L (2014). Effectiveness of a novel spontaneous carvacrol nanoemulsion against Salmonella enterica Enteritidis and Escherichia coli O157: H7 on contaminated mung bean and alfalfa seeds. International journal of food microbiology, 187, 15-21.
6. Maté J, Periago PM, Palop A (2016). Combined effect of a nanoemulsion of d-limonene and nisin on Listeria monocytogenes growth and viability in culture media and foods. Revista de Agaroquimica y Tecnologia de Alimentos, 22,146-152.
7. Settanni L, Palazzolo E, Guarrasi V, Aleo A, Mammina C, Moschetti G, Germanà MA (2012). Inhibition of foodborne pathogen bacteria by essential oils extracted from citrus fruits cultivated in Sicily. Food Control, 26, 326-330.
8. Xiao D, Gömmel C, Davidson P M, Zhong Q (2011). Intrinsic Tween 20 improves release and antilisterial properties of co-encapsulated nisin and thymol. Journal of Agriculture and Food Chemistry, 59, 9572–9580.
9. Zahi MR, Liang H, Yuan Q (2015. Improving the antimicrobial activity of d-limonene using a novel organogel-based nanoemulsion. Food Control, 50, 554-559.
10. Zhang Y, Liu X, Wang Y, Jiang P, Quek S (2016). Antibacterial activity and mechanism of cinnamon essential oil against Escherichia coli and Staphylococcus aureus. Food Control, 59, 282-289.