Impact of antimicrobial-loaded emulsion system stabilized with gum arabic on food-borne pathogens

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Abstract: With increased awareness among consumers on human health and nutrition, there is a drastic shift in eating patterns and increased consumption of fruits and vegetables. However, food safety remained a prime concern among authorities and regulatory bodies with perishable commodities being easily prone to microbial contamination and related food-borne disease outbreaks. Use of synthetic preservatives and chemical treatments has proven to be expensive and pose serious health hazards. Thus, one alternative approach could be the use of antimicrobial emulsion-based edible coating over fresh-cut fruits to inactivate microorganisms and obtain enhanced shelf life. The key aspect of this research is to formulate and characterize essential oil loaded emulsions stabilized with gum arabic. Briefly, formulated samples were subjected to ultrasonic emulsification at 40% amplitude for 3 min duration, and the emulsion-based edible coating was applied over fresh-cut papaya. The antimicrobial efficacy of model antimicrobials carvacrol and cinnamaldehyde were tested alone and in combination against Gram-positive bacteria. The study demonstrated that concentration of antimicrobial compounds used in combination work synergistically. Secondly, use of antimicrobial loaded emulsion as the delivery vehicle would yield enhanced shelf life against food-borne pathogens.

Keywords: Food safety, foodborne pathogens, Gram-positive bacteria

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Introduction

- With increased awareness among consumers on human health and nutrition, there is a drastic shift in eating patterns and increased consumption of fruits and vegetables. However, food safety remains a prime concern among authorities and regulatory bodies with perishable commodities being easily prone to microbial contamination and related food-borne disease outbreaks.
- Use of synthetic preservatives and chemical treatments has proven to be expensive and pose serious health hazards. Thus, one alternative approach could be use of antimicrobial emulsion-based edible coating over fresh-cut fruits to inactivate microorganisms and obtain enhanced shelf-life.
- Ultrasonication is used to deliver high frequency sound waves possessing frequency greater than 20 kHz for the production of mechanical vibrations in the surrounding medium. This leads to formation of cavities through acoustic cavitation phenomenon.
- The cavities collapse leading to the generation of immense energy that transits in the surrounding medium in the form of shock waves. This leads to reduction in the size of the coarse emulsions to micro and nanometric range.

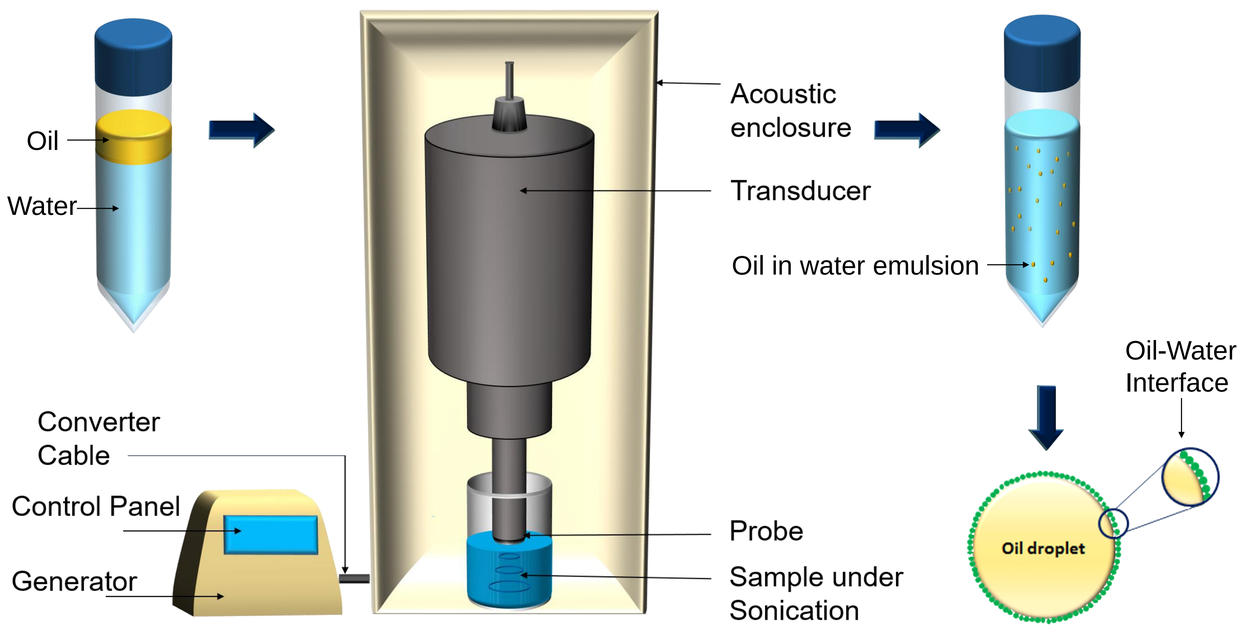


Fig. 1 Schematic diagram of probe ultra sonicator and emulsification process

Methods

- The key aspect of this research is to formulate and characterize essential oil loaded emulsions stabilized with gum arabic (1% w/v).
- The oil-in-water emulsions containing different amounts of carvacrol and cinnamaldehyde in the oil phase and 50 mM sodium acetate buffer, pH 5.5 as aqueous phase were produced.
- Formulated samples were subjected to ultrasonic emulsification at 40% amplitude for 5 min duration and emulsion-based edible coating was applied over fresh-cut papaya.
- Droplet size of Emulsion and ζ-Potential of Emulsion: Malvern Zetasizer Nano ZS dynamic light scattering particle size analyser.
- The antimicrobial efficacy of model antimicrobials carvacrol and cinnamaldehyde was tested alone and in combination against Gram-positive *Listeria innocua*.
- Checker board method was adopted for determination of interactive inhibition.

Results and Discussion

Droplet size of Emulsion

- The average particle size of formulated emulsions ranged from 180 nm to 260 nm

ζ - Potential of Emulsion

- ζ-potential values of the formulations were observed to be greater than -16 mV

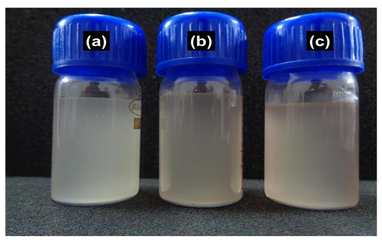


Fig. 2 Emulsions prepared from essential oils (a) carvacrol oil (b) combined essential oils (c) cinnamaldehyde oil

Table 1. Impact of mass ratio of carvacrol and cinnamaldehyde on resulting particle size

Carvacrol: Cinnamaldehyde (w:w)	Z-average diameter (nm)	ζ – Potential (mV)
1:0	260.5 ± 5.3	-16.6 ± 1.3
1:1	234.8 ± 9.2	-18.2 ± 0.26
0:1	180.4 ± 6.5	-17.5 ± 0.6

[a] Data represent mean± standard deviation (n=3)

Effect of storage stability on emulsion

- For commercial application of emulsions based products, it is requisite that the prepared formulations maintain the particle size throughout the designated shelf life.
- It was observed that all the preparations after 30 days of storage maintained the particle size with a variation of less than 4.94%, indicating greater stability.
- The relative increase in the mean particle diameter of the samples can be attributed to coalescence of the droplets.

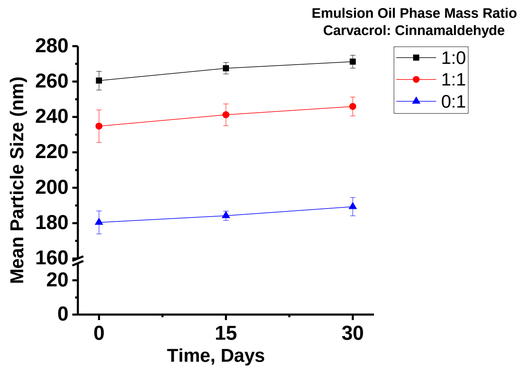


Fig. 3 Dependence of mean droplet diameter of emulsions after 0, 15 and 30 days of storage at 25°C. The standard deviation was indicated in the form of error bars (n=3)

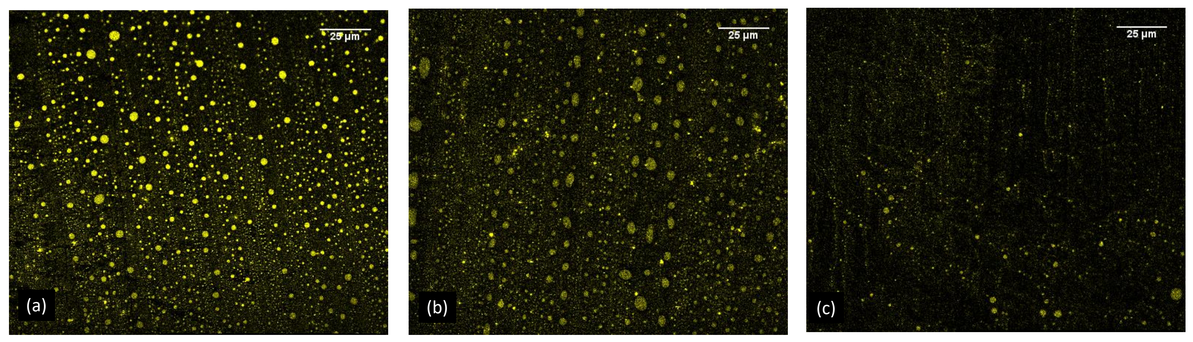


Fig. 4 Confocal images of emulsions prepared from (a) carvacrol oil (b) combined essential oils and (c) cinnamaldehyde oil. Scale bar 25 µm

Antimicrobial susceptibility test

- The lowest antimicrobial concentration that would inhibit the visible growth of the representative microorganism is referred to as the Minimum inhibitory concentration (MIC) value for individual and combined antimicrobial agents
- To investigate the interactive inhibition of antimicrobial combinations checkerboard method was employed and combined with calculation of fractional inhibitory concentration (FIC).
- The FIC was calculated as $FIC_A + FIC_B$, where FIC_A = (MIC_A of the combination/MIC_A alone) and FIC_B = (MIC_B of the combination/MIC_B alone). The results were interpreted as synergy (FIC < 0.5), addition (0.5 < FIC<1), indifference (1< FIC< 4) or antagonism (FIC > 4).

BHI broth as model testing system

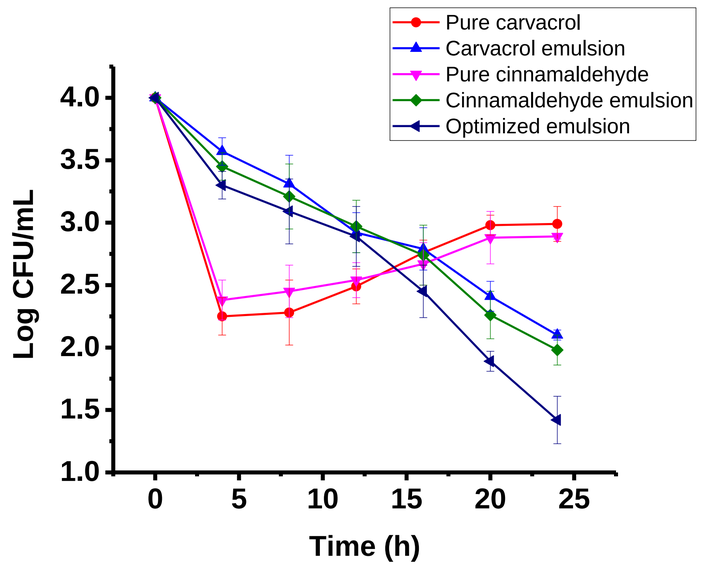
Table 2. Minimum inhibitory concentration (MIC) of carvacrol and cinnamaldehyde against tested Gram-positive model bacteria [a]

Tested bacteria	MIC alone		MIC combined		FIC
	Carvacrol (µg/mL)	Cinnamaldehyde (µg/mL)	Carvacrol (µg/mL)	Cinnamaldehyde (µg/mL)	
Listeria innocua	750	700	200	150	0.480

[a] Results are the mean of three different experiments

Fresh cut fruit (Papaya) as model testing system

- The optimized nanoemulsion was tested on model microorganisms. Growth curve was studied to observe the antimicrobial effect of optimized emulsion on the survival of *Listeria innocua*
- Model bacteria was found to be susceptible.



- For pure essential oils, strong inhibitory effect was noticed during the initial 4 h.
- With quick decrease in concentration of pure essential oils, the bacterial cell concentration increased to higher levels.
- Long term inhibition was noticed for essential oils based emulsions against *Listeria innocua*.
- Similarly, impact of emulsion based edible coating on real-food system such as fresh-cut papaya was investigated and observed to reduce the *Listeria innocua* concentration by 3.5 $\log_{10}$ CFU/g.

Fig. 5 Antimicrobial activity of pure carvacrol oil, pure cinnamaldehyde oil and optimized essential oil based emulsion against *Listeria innocua* with papaya as model testing system. Mean values are shown with error bars of standard deviation (n=3)

Conclusion

- The study demonstrated that concentration of antimicrobial compounds used in combination work synergistically.
- Use of antimicrobial loaded emulsion as delivery vehicle would yield enhanced shelf life of fresh-cut papaya against tested bacteria.

References

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