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Antibacterial Activities of Thymol, Eugenol and Nisin Against Some Food Spoilage Bacteria

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ABSTRACT

Antibacterial activities of nisin, thymol and eugenol were assayed against four strains of common spoilage bacteria, so as to introduce into food packaging material for shelf-life benefit. Those bacterial strains included *E. coli* ATCC 25922 DMST 4212, *S. aureus* ATCC 25923 DMST 8840, *B. cereus* ATCC 11778 DMST 5040 and *L. monocytogenes* DMST 17303 were used for this study by agar well diffusion test on nutrient agar. The active inoculum size between 10^5-10^6 cfu/g of each strain was inoculated into the sterile warmed liquefied medium, prior to plate pouring. After solidified, holes of 5 mm in diameter were punctured using a sterilized cork borer # 2. Then, an individual of 40 µl two-fold serial dilution from each agent was applied for MIC determination with experimental design for three replicates. Results of clear zones were reported after 24 hr incubation at 37 °C. It was found that MICs for thymol, eugenol and nisin was between 3-5, 8-11 and 4-23 mg/ml, respectively, depending on each bacterial strain tested. This was concluded that thymol appeared to be the most preferable agent for future research in active food packaging development, especially in term of antimicrobial benefit.

Key words: thymol, eugenol, nisin, antimicrobial

INTRODUCTION

The consequences of quality loss of food product caused by microorganisms are consumers risk, due to the presence of microbial toxins and pathogens as well. Food antimicrobials are compounds added to or present in foods to retard microbial growth or kill microorganisms. However, today consumers'demand favor consumption of natural foods which contain fewer synthetic additives, this made the use of natural antimicrobials in food popular (Davidson, 1997).

Plant essential oils are a potentially useful source of antimicrobial compounds that have been shown to posses distinct antimicrobial activities against many foodborne pathogens. Thymol and eugenol are both natural preservative substances that are not toxic when applied at an appropriate amount in food products. Thymol is the major essential oil component in oregano and thyme (Davidson, 1997) and is also active against several species of bacteria such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus subtilis*, *B. cereus*, *Escherichia coli*, *Salmonella enterica*, *S. typhimurium*, *Pseudomonas aeruginosa* and *Campylobacter jejuni* (Sivropoulou *et al*., 1996; Friedman *et al*., 2002) and fungi (*Rhizobium leguminosarum*) (Sivropoulou *et al*., 1996). Eugenol is the major essential oil component in clove, cinnamon,

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allspice and basil (Davidson, 1997). Several reports have shown the antimicrobial effect of eugenol against several species of bacteria, i.e. *L. monocytogenes*, *B. cereus*, *C. jejuni*, *E. coli* 0157:H7, *S. enterica*, *Aeromonas hydrophila*, *S. typhimurium* and *Enterobacter aerogenes* (Thoroski *et al*., 1989; Kim *et al*., 1995; Wendakoon and Sakaguchi, 1995; Hao *et al*., 1998; Friedman *et al*., 2002) and fungi, i.e. *Aspergillus* spp. and *Penicillium* spp. (Bullerman *et al*., 1977; Vazquez *et al*., 2001).

Among various natural antimicrobial agents, nisin which is a polypeptide produced by *Lactococcus lactis* subsp. *lactis* has been most widely studied for its activities. Because it is produced by lactic acid bacteria, that often found in human's digestive tract and recognized as probiotic (good microorganism). Nisin was effective against a wide range of spoilage and pathogenic gram-positive bacteria (Han, 2005). Many recent studies reported that nisin inhibited growth of gram-positive bacteria, such as *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Lactobacillus plantarum*, *Micrococcus luteus*, *Micrococcus flavus* and *Brochothrix thermospacta* (Padgett *et al*., 1998; Siragusa *et al*., 1999; Cutter *et al*., 2001; Periago and Moezelaar, 2001; Singh *et al*., 2001; Kim *et al*., 2002; Cha *et al*., 2003; Pranoto *et al*., 2005).

The objective of this study was to investigate antibacterial properties of nisin, thymol and eugenol against bacterial strains generally encountered in food spoilage. The MICs of the antibacterial agent were also determined.

MATERIALS AND METHODS

Microorganisms tested

Gram-negative *Escherichia coli* ATCC 25922 DMST 4212, gram-positive *Staphylococcus aureus* ATCC 25923 DMST 8840, gram-positive *Bacillus cereus* ATCC 11778 DMST 5040 and gram-positive *Listeria monocytogenes* DMST 17303 were obtained from the Department of Medical Sciences, Ministry of Public Health, Thailand.

Antibacterial agents for bactericidal assay

Thymol 99% FCC and eugenol 98% natural oils were obtained from Sigma-Aldrich, Inc. (USA). A two-fold serial dilution of thymol and eugenol were prepared by diluting with sterile 75% (v/v) dimethyl sulfoxide (DMSO) to achieve a decreased concentration range of 40 mg/ml to 0.0195 mg/ml.

Nisin (1,000,000 IU/g) was obtained from Sigma-aldrich, Inc. (USA). A two-fold serial dilution of nisin was prepared by diluting with sterile 0.02 M HCl to achieve a decreased concentration range of 40 mg/ml to 0.0195 mg/ml.

Determination of minimum inhibitory concentrations (MICs)

The MICs were determined by the agar well diffusion method (Iroegbu and Nkere, 2005). Nutrient agar along with 1 ml of inoculum 10^5-10^6 colony forming unit per ml of each bacteria tested was poured into sterile plates, prior to set for solidification. Then, four holes of each agar was made, by using a steriled cork borers (No.2, diameter 5 mm). A forty microliters of two-fold serial dilitions of the prepared antibacterial agent was added into each well, along with controls from sterile 75% (v/v) DMSO and 0.02 M HCl. All tested plates were incubated at 37 °C for 24 hours. The minimum concentration of each extract showing a clear zone larger than 7 mm was taken as the MICs (Moreira *et al*., 2005). The experiments were carried out in triplicate.

RESULTS AND DISCUSSIONS

All strains showed similar sensitivity of thymol and eugenol. However, nisin exhibited no antibacterial effect on *Escherichia coli* (Table 1).

Table 1 shows clear zone of thymol, eugenol and nisin against *L. monocytogenes*, *S. aureus*, *B. cereus* and *E. coli* at Minimum Inhibitory Concentration (MICs) and Table 2 shows the MICs of thymol, eugenol and nisin against these four bacterial strains. Thymol and eugenol were active against all four strains tested. The MICs were 3 to 5 mg/ml and 8 to 11 mg/ml for thymol and eugenol respectively, depending on bacterial strain applied. *Escherichia coli* showed the highest sensitivity to thymol and eugenol compared with the other organisms. The results were similar to the reports of Friedman *et al*. (2002) and Olasupol *et al*. (2003). They found that thymol and eugenol were active against *L. monocytogenes, E. coli*, *C. jejuni*, *S. enterica* and *S. T*yphimurium, whereas thymol was more active than eugenol. Figure 1-4 exhibit clear zone of thymol against all tested bacterial strains at various MICs values.

Thymol and eugenol exhibited the antibacterial effect against the gram-negative bacteria. An important characteristic of thymol and eugenol was their hydrophobicity. This property enable them to penetrate the lipopolysaccharide of the gram-negative bacterial cell membrane, and disturbed the cell structures. Therefore, leakage of ions and other cell contents occured (Burt, 2004). The results showed that nisin inhibited the gram-positive bacteria, corresponding to previous studies (Padgett *et al*., 1998; Pranoto *et al*., 2005). The C-terminal region of nisin binded to the cytoplasmic membrane of vegetative cells, penetrated into the lipid phase of the membrane,

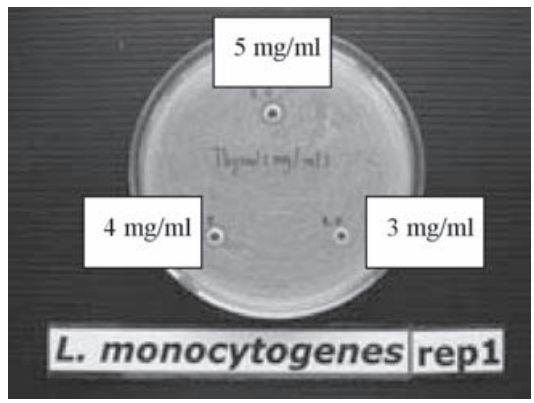

Figure 1 Clear zone of thymol against *Listeria moocytogenes* at the MIC = 4 mg/ml.

Table 1 Antibacterial activity of thymol, eugenol and nisin against tested bacterial strains.

Microorganisms	Clear zone (mm)[a]		
	Thymol	Eugenol	Nisin
L. monocytogenes	7.1 ± 0.6	7.4 ± 0.5	7.4 ± 0.5
S. aureus	8.5 ± 0.8	7.3 ± 0.4	7.1 ± 1.0
B. cereus	8.0 ± 0.8	7.0 ± 0.4	7.2 ± 0.4
E. coli	7.6 ± 0.9	7.8 ± 1.3	NG[b]

[a] Mean ± Standard deviation (n=3)

[b] NG indicates no antibacterial effect on visible growth.

Table 2 Minimum inhibitory concentration (MICs) of thymol, eugenol and nisin.

Microorganisms	Minimum inhibitory concentration (mg/ml)[a]		
	Thymol	Eugenol	Nisin
L. monocytogenes	4.00	11.00	4.00
S. aureus	5.00	8.00	5.00
B. cereus	5.00	9.00	23.00
E. coli	3.00	8.00	NG[b]

[a] MIC values were determined by adding antibacterial agents into nutrient agar.

[b] NG indicates no antibacterial effect on visible growth.

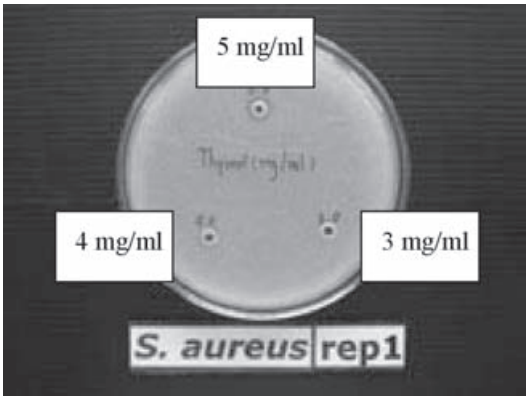

Figure 2 Clear zone of thymol against *Staphylococcus aureus* at the MIC = 5 mg/ml.

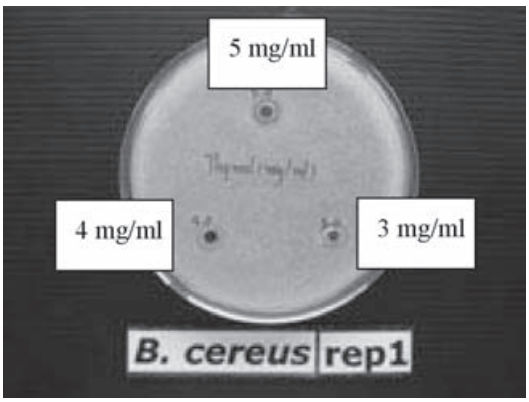

Figure 3 Clear zone of thymol against *Bacillus cereus* at the MIC = 5 mg/ml.

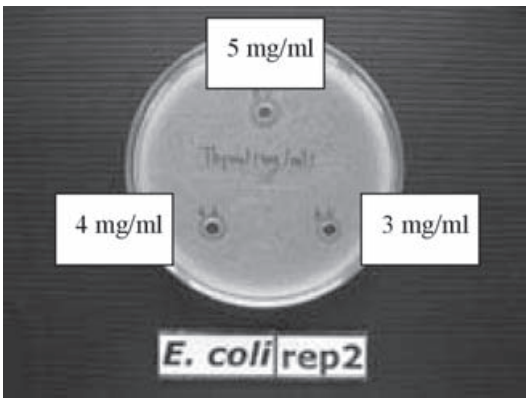

Figure 4 Clear zone of thymol against *Escherichia coli* at the MIC = 3 mg/ml.

and formed the pores, which allow the efflux of potassium ions, ATP and amino acids. This resulted in a dissipation of the proton motive force and hence cell death (Periago and Moezelaar, 2001). As the cell wall of the gram-negative bacteria is more complex, although thiner, than that of the gram-possitive bacteria, it hindered nisin to penetrate in the cell membrane of bacteria (Padgett *et al*., 1998 and Pranoto *et al*., 2005).

CONCLUSION

This study showed that thymol and eugenol could inhibit all bacterial strains tested, of which they generally encountered in food spoilage. However, nisin exhibited no antibacterial effect on *Escherichia coli*. It indicated that thymol had higher antibacterial activity than eugenol and nisin and could be used as the antibacterial agent to incorporate in packaging materials.

ACKNOWLEDGEMENTS

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