

Effects of essential oils on *Staphylococcus aureus*, *Escherichia coli* and *Listeria monocytogenes* in a food packaging system



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Determination of bacterial resistance to antimicrobials is an important part of the development of new and active packaging materials. Essential oils (EOs) exhibit antimicrobial activity against specific microorganisms and are considered as a safe alternative to traditional preservatives in foods. A laboratory screening was carried out to test how EOs can be used in a packaging system.

Material and methods

The inhibitory effect of EOs was examined on Gram negative *Escherichia coli*, and on Gram positive *Staphylococcus aureus* and *Listeria monocytogenes*. The tested oils were thyme, clove, rosemary, tea tree and cold pressed rosehip and grape seed oils. Sterile filter discs with 6 mm diameter were added 10 µl of oils and placed on the agar surface inoculated with the test organism. The disk diffusion method was used as a screening method on Mueller-Hinton agar (Fig. 1). Effects of volatile components were tested in sealed petri dishes with a disc separated from the agar (37 °C in 24 and 48 h).



Figure 1. Principle presentation of disc agar diffusion (left) and volatile vapour diffusion method (right).

The most effective EO's were used to examine effects of volatile components inside a simulated packaging system. A sliced core sample of a minced fish product were inoculated with *L. monocytogenes* (10⁵ cfu/g) and effects of volatiles compounds were tested in sealed petri dishes (Fig 2).



Fig 2. A minced fish products (left) were cut in slices (3 cm diameter.), inoculated with *L.monocytogenes* and placed in a closed petridish together with discs saturated with Thyme oil (middle). Volatile oil components was spread in the closed package (right)

Results and discussion

There were insignificant inhibition zones using extracts from grape seeds and rosehip seeds. For the disk diffusion method the largest inhibition zone (diameter) were found for thyme (33.8 – 69.5 mm), then clove (16.0 – 29.0 mm) and tea tree (16.0 – 25.0 mm), while rosemary gave little inhibition (Table 1). The same inhibition pattern were seen for both *S. aureus*, *E. coli* and *L. monocytogenes*.

Table 1. Mean inhibition zone diameter (mm) with 6 mm disc size of four parallel MH plates except *L. monocytogenes* (two parallel plates) after 24 h incubation

EOs	Quantity of EOs	Direct contact - Disc diffusion method			Vapour phase method		
		<i>S. aureus</i>	<i>E. coli</i>	<i>L. monocytogenes</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>L. monocytogenes</i>
Thyme	10 µl	67.2 ± 8.8	33.8 ± 2.0	69.5 ± 6.4	56.8 ± 6.9	28.3 ± 1.3	48.5 ± 4.9
Tea tree	10 µl	25.0 ± 4.2	16.0 ± 1.2	22 ± 2.8	n.z.	n.z.	n.z.
Clove	10 µl	29.0 ± 2.4	16.0 ± 2.7	28.5 ± 2.1	14 ± 2.6	n.z.	n.z.

n.z. = no zone

Inhibition with *L. monocytogenes* on minced fish

Thyme gave the largest antibacterial effect on *L. monocytogenes* when tested in the volatile laboratory diffusion test (Fig 3). This indicated that thyme could be effective against *Listeria* in a closed package with a fish product.

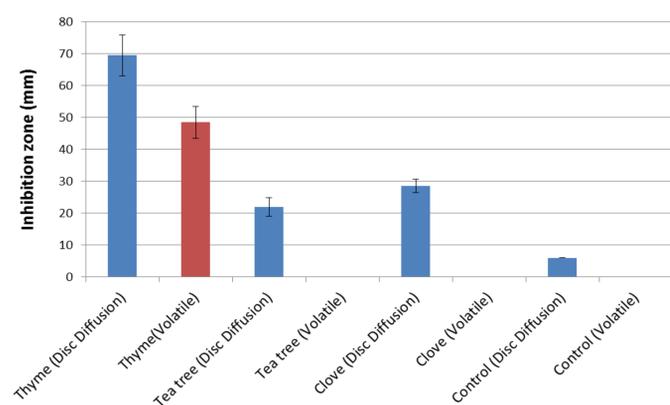


Figure 3 Inhibition zones of of EO's on *L. monocytogenes* in disc diffusion tests and volatile tests .

When 10 and 30 µl of thyme were exposed to minced fish samples inoculated with *L. monocytogenes* in a simulated package, no significant effect were observed. (Fig 4). The sample plates were incubated in plastic bags at a temperatures of 37°C for 3 days.

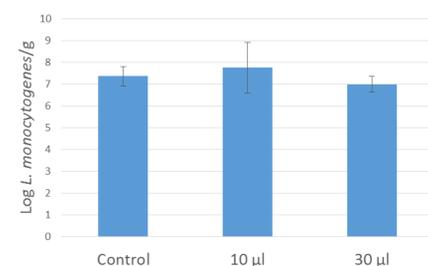


Figure 4 Similar levels of *L. monocytogenes* was found in packages with 0, 1 (10 µl thyme) and 3 (30 µl thyme) discs.

The bacteria are protected on the food surface and higher concentration of EOs was needed compared to the results found in the laboratory agar test system, to inhibit the growth.

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Conclusions

- The disc diffusion and volatile method are suitable methods for screening EOs.
- Thyme, clove and tea tree oils are the most effective antibacterial oils.
- 10 µl of thyme oil is an effective antimicrobial EOs against *L. monocytogenes* in the disc diffusion and volatile test.
- When *L. monocytogenes* was inoculated (10⁷ cfu/g) on a minced fish surface, 10 – 30 µl thyme had no detectable antimicrobial effects.