Antimicrobial efficacy of lemongrass (*Cymbopogon citratus*) and Fingerroot (*Boesenbergia pandurata*) essential oils against foodborne pathogens

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Introduction

The consumption of ready-to-eat meat products such as sausages, meatloaf, dried meat and cakes is increasing mainly in developing countries (Heinz and Hautzinger, 2010). However, the risk of contamination with food-borne pathogens from poorly processed and stored meat products, such as *salmonellosis*, *Escherichia coli* and other pathogens pose a great health hazard that needs to be controlled. Presently, these pathogens are being controlled with the aid of synthetic or natural preservatives. However, concerns about the safety of chemical additives are on the rise in past recent years. As a consequence, consumers are progressively demanding the use of natural products as alternative for synthetic preservatives (Balciunas et al., 2013). Plants are a source of bioactive molecules and have been widely used both traditionally and commercially to increase the shelf-life and safety of foods (Sasidharan et al., 2008). Thus, this study investigates the potential of essential oils (EOs) of Lemongrass and Fingerroot as a natural preservative to control four common foodborne pathogens *in vitro*.

Objectives

The main objective was to identify *in vitro* antimicrobial efficacy of two EOs against four different food-borne pathogens

Particular objectives were:

1.To determine the minimal inhibitory concentration (MIC) of EOs

2.To analyse *in vitro* inhibition over time in different conditions

Materials and methods

Essential Oils

The EOs of Lemongrass (*Cymbopogon citratus)* and Fingerroot (*Boesenbergia pandurata)* were used in this work. Both EOs were obtained from BOTANICESSENCE Essential Oils, Thailand. The EOs were certified by Ecocert SA (F32600).

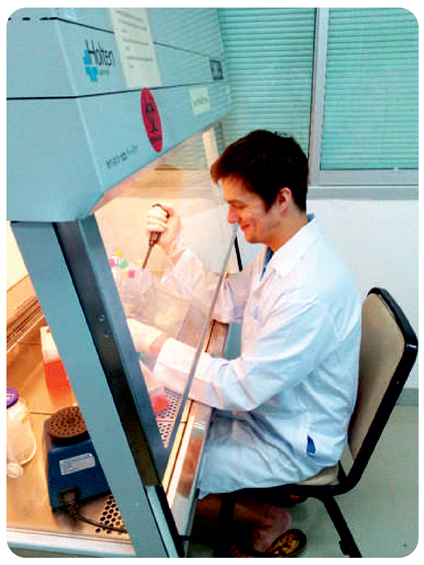

Figure 1. Samples preparation, HatYai, Thailand.

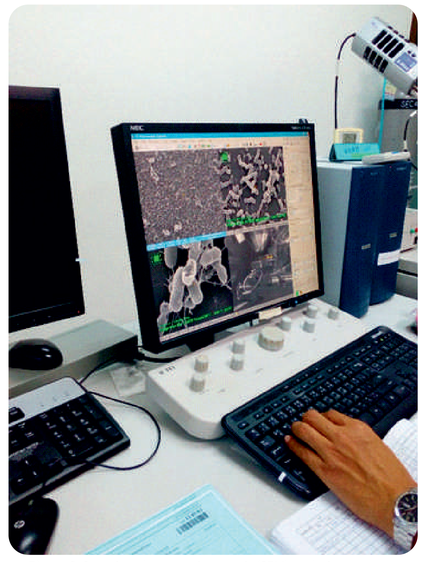

Figure 2. Scanning electron microscopic (SEM) analysis of damaged cells.

Bacterial strains and culture conditions

Tested pathogenic bacteria were comprised of *Salmonella enteritidis (DMST), Escherichia coli* (DMST), *Listeria monocytogenes* (F2365), and *Staphylococcus aureus* (DMST). These microorganisms were chosen as they are commonly associated with the spoilage of refrigerated foods. All species were supplied by the Faculty of Agro-Industry, Prince of Songkla University (Hat Yai, Thailand). The stock cultures of bacterial strains were prepared overnight in brain heart infusion broth (BHIB) at 37 °C and then they were streaked on the brain heart infusion agar (BHIA) and incubated for 24 hours at (37 °C). The cultures were kept under refrigerated conditions and were subcultured after every ten days.

Table 1. MIC and MBC of the lemongrass EO against tested organisms (%, v/v)

Tested organism	MIC 4°C	MBC 4°C	MIC 25°C	MBC 25°C
Listeria Monocytogenes (LM)	0.03	0.13	0.13	0.50
Staphylococcus Aureus (SA)	0.03	0.06	0.06	0.50
Escherichia Coli (EC)	0.03	0.25	0.06	1.00
Salmonella Montevideo (SM)	0.03	0.13	0.13	0.50

Table 2. MIC and MBC of the fingerroot EO against tested organisms (%, v/v)

Tested organism	MIC 4°C	MBC 4°C	MIC 25°C	MBC 25°C
Listeria Monocytogenes (LM)	0.25	0.50	0.50	2.00
Staphylococcus Aureus (SA)	0.06	0.50	0.50	1.00
Escherichia Coli (EC)	0.13	0.50	0.50	1.00
Salmonella Montevideo (SM)	0.13	1.00	0.50	0.50

Microbial assay

The broth macrodilution method was used to determine he MICs and MBCs of oils as explained by Hammer et al. The MIC was defined as the lowest concentration of the test compound to inhibit the growth of microorganisms and the MBC was defined as the lowest concentration of the test compound to kill the microorganisms.

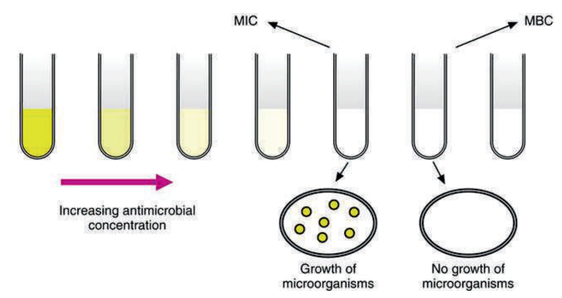

Figure 3. The broth macrodilution method.

Inhibition over time

The sterile tubes with BHI broth (5 ml) were inoculated with 2 different EOs at concentrations MIC, 2xMIC, 4xMIC and with 0.5 % (v/v) of tween-80. Then 100 µL of active inoculums of each bacteria (10^6 CFU/ml) was added. Sampling for viable cells were carried out at day 0, 1, 3 and 5, at two different storage temperatures (4 °C and 25 °C). The viable plate counts were monitored as follow: 50µL sample of each treatment was spread on the surface of BHIA and the colonies were counted after incubation at 37 °C for 24 h. At each assay time, controls without EOs were also tested.

Results and discusion

Both EOs were found effective against all four tested organisms. Gram positive organisms (*S. aureus, L. monocytogenes*) showed similar sensitivity to EOs as gram negative organisms (*E. coli, S. enteretidis*). Similar observations were made by Onawunmi and Ongulana et al. The antibacterial activity was found progressively increasing with the increase in concentration of oil. On the other side, the antibacterial activity of both EOs was lower in the case of higher temperature (25°C) in all tested organism. As can be seen at table 1 and table 2, the MIC at 4°C varies from 0.03 to 0.25% and at 25°C from 0.06 to 0.50% respectively. Lemongrass essential oil showed higher efficacy against all tested organism in both temperatures.

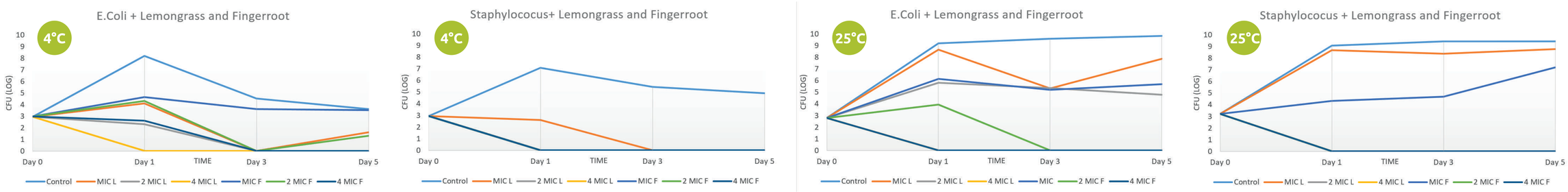

Figure 4.-7. Inhibition over the time (CFU – colony forming units, L - lemongrass, F – fingerroot)

Conlusion

Lemongrass and fingerroot EOs produced bacteriostatic effect against *Escherichia coli, Listeria monocytogenes, Staphylococcus aureus* and *Salmonella enteritidis in vitro*. From the present study it is clear that lemongrass EO is more efficient than fingerroot EO against all tested organisms. Both EOs showed inhibitory effect even on very low concentrations, hence both the spices provide a potential for their use as natural preservatives.

Refences:

Baliunac M.E., Martinez C.A.F., Todorov D.W. et al. Novel biotechnological applications of bacteriocins: A review. Food control. July 2013. 32(1):134-142. Hammer K.A., Carron C.F., Relay T.V. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol. 1999. 86: 985-90. Heinz V., Hautzinger P. Meat processing technology for small-to medium-scale producers. 2010. Acessed online at http://www.fao.org/docrep/010/ai407e/AI407E08.htm on 12th July 2017. Onawunmi G.O., Yesiak W.A.B, Ongulana E.O. Antibacterial constituent in the essential oil of Cymbopogon citratus. J Ethanopharmocol 1984; 12(3): 279-86. Sasidharan S., Zakaria Z., Latchmanan Y.I., Suryani S. Antimicrobial Activities of Psophocarpus tetragonolobus (L.) DC Extracts. Article in Foodborne Pathogens and Disease. June 2008. 5(3):303-9.