Doctoral Thesis

Development of hurdle technology using plant extracts for control of foodborne pathogens

(Summary)

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Despite many countries installing food safety intervention measures, foodborne outbreaks including those caused by salmonella and listeria are on the rise. Use of synthetic preservatives which is one such common approach, does not satisfy consumers because of safety and nutritional issues regarding food containing chemical preservatives such as sodium benzoates and potassium sorbates. Consumers are increasingly aware of health implications associated with consumption of food containing some synthetics or conventional preservatives as such demand food which is safer. This has been food industries driving force towards natural preservatives which includes dietary herbs and spices which appeal to consumers because have already been part of human diet since antiquity and have Generally Recognized as Safe (GRAS) substances according to Food and Drug Administration (FDA). However, dietary herbs and spice extracts exhibit activity at a concentration which may not be economical to application. Also, higher levels impact negatively on the sensory attributes of food and water.

Against this background, there is need to find ways of reducing herbs and spice extracts effective levels in order to widen their application. This study aimed at identifying dietary herbs and spices with antibacterial activity and then improve the activity by combining with other GRAS antimicrobials against foodborne pathogens. Outbreaks concerning vibrios are on the rise. This study focused on control of *Vibrio cholerae* (*V. cholerae*)

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined by agar and broth dilution assays respectively. Those extracts that demonstrated lower (stronger activity) MIC and MBC were selected for time-kill experiment to verify killing effect and comprehend bactericidal pattern. Additionally, combinatorial study was done with selected extracts in order to create hurdle effect.

Ethanolic extracts of lemon eucalyptus exhibited strong antibacterial activity with a MIC of 0.025 % followed by curry plant with a MIC of 0.05 % against Gram-positive bacteria tested. Lemon eucalyptus and curry plant showed a MBC of 0.1 % against Gram-positive bacteria. MIC of lemon eucalyptus reduced from 0.025-0.0125 % in the presence of glycine 0.2 % at pH 5.5. Activity of curry plant was improved from MIC of 0.05 % to 0.0125-0.025 % with glycine 0.2 % at pH 5.5. MBC of lemon eucalyptus and curry plant was enhanced from

0.1% to 0.025-0.05 % in the presence of glycine 0.5 % at pH 5.5. Ethanolic extracts of clove, lemon eucalyptus, licorice, rosemary, sage and thyme were active against *V. Cholerae* with a MIC of 0.2 % except thyme (0.4 %). Activity of plant extracts was amplified through incorporation of sodium acetate. Mixture of plant extract with sodium acetate (0.4%) at pH 7.0 reduced MICs of clove, lemon eucalyptus, rosemary, sage and thyme to 0.025 % and that of licorice to 0.05 %. At pH 6.4, combinations were more effective reducing MICs of clove, lemon eucalyptus, rosemary and sage to 0.0125 % with sodium acetate 0.2 %. Plant extracts and sodium acetate at pH 7.0 resulted in inhibitory activity while at pH 6.4 vibriocidal was observed. At pH 7.0, the mixture resulted in additive effect, nevertheless, at pH 6.4, synergy with Fractional Inhibitory Concentration (FIC) index of 0.375-0.5 was observed. In contaminated water, time kill curves confirmed bactericidal effect in a dose and time dependent manner. Extracts of licorice and sage showed significant reduction in viable cell count after exposure of 4-8 h. Within 4-8 h, extract of sage eliminated *V. cholerae*.

Lemon eucalyptus demonstrated most potential and broad inhibitory activity. Curry plant exhibited specificity against Gram-positive bacteria while rosemary was more specific on *V. cholerae*. Plant extracts showed high potency (low MIC) when NaOAc or glycine was incorporated. More amplified activity was established when plant extract-NaOAc/glycine mixture was at mildly acidic pH. The obtained data was evaluated to have a combinatorial effect which could be a potential candidate for gentle but effective ways of controlling foodborne bacterial pathogens (hurdle technology). In contaminated water licorice and sage exerted significant anti-vibrio activity indicating a potential for use as additives where reduction in bacterial count or preservation is desired. More importantly, is definite exposure time established in this study to achieve complete disinfection. This may have a positive implication in control of *V. cholerae* in water which serves as one of main vehicles for transmission. Furthermore, no off odour was detected with both extracts in water after incubation period. This suggests that sage and licorice extracts could be applied in water as decontaminants. Further study may utilize these findings to examine activity of the combinations in food and water.