学 位 論 文 の 要 旨

論文題目 Application of ethanolic plant extract combined with other hurdles in controlling *Escherichia coli*

（植物エタノール抽出液と他の複合ハードルによる大腸菌の制御）

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Escherichia coli O157:H7 is a major foodborne pathogen, which causes hemorrhagic colitis, hemolytic uremic syndrome. Besides, it has also been reported to survive in acidic environments also in high levels of salt. In controlling *E. coli* O157:H7, the use of a single hurdle might require a high concentration of antimicrobial agents or severe processing. Nowadays, the application of chemical preservatives is wildly not accepted due to concern about their harmful effects on human health. Thus, consumer demand has focused on using natural antimicrobials or antimicrobials as recognition as GRAS (Generally Recognized as Safe) by FDA. Thus, this study aimed to investigate the hurdle technology application of ethanolic plant extracts with other hurdles to control the growth of *E. coli.*

In the first study, the antimicrobial activities of 22 plant extracts against pathogenic and non-pathogenic strains of *E. coli* were observed. This study also determined the potential hurdle technology application of plant extracts with glycine and NaOAc under neutral and mildly acidic pH conditions. The results showed that only clove and rosemary extracts exhibited antimicrobial activity against *E. coli* with diameter of inhibition zone (DIZ) values of 12.25 mm and 11.75 mm, respectively. Thus, only clove and rosemary extracts were observed for their minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) and studied for the chemical component by GC-MS. Clove extract inhibited the antimicrobial activity against *E. coli* with a bactericidal effect with both MIC and MBC of 0.4%, while MIC and MBC of rosemary extract were 0.6% and 0.8%, respectively. The main components of clove and rosemary extracts were eugenol, and glycidol, respectively. The effect of glycine or NaOAc on the antimicrobial activity of plant extracts against *E. coli* in neutral and mildly acidic medium was also observed. It was

shown how various interactions ranging from absent to additive effects were observed between the plant extracts and glycine or NaOAc. Combined effects of 0.2% clove extract under neutral condition with 0.1% NaOAc or 1.0% glycine demonstrated additive interactive antimicrobial activity, thus FIC indices 0.53 or 0.75, respectively. Under pH 5.5, supplementations of 0.1% NaOAc with 0.1% clove or 0.2% rosemary extracts showed additive interaction with FIC indices 0.53-0.70.

In the second study, the development of hurdle technology in controlling pathogenic *E. coli* O157:H7 by using plant extracts with glycine and/or NaOAc under neutral and mildly acidic nutrient broth (NB) and phosphate-buffered saline (PBS) under different incubation temperature (15 °C and 35 °C) was studied. Treatments in NB under 15°C, and neutral pH, the combination of 0.2% clove extract + 2% glycine + 2% NaOAc showed the highest efficacy against *E. coli* with 3 logs reduction after 48 h, while under mildly acidic conditions, treatments with 0.2% clove extract + 2% NaOAc reduced the bacterial population by approximately 3 log units after 24 h. Under 35 °C incubation, conditions the two combined factors (0.2% clove extract with 2% glycine or 2% NaOAc) achieved about 3 log reduction after 12 h, while treatments with 0.1% clove extract + 2% glycine + 2% NaOAc completely inhibited *E. coli* after 24 h in neutral media. Under pH 5.5, the combination of 0.2% clove extract with 2% NaOAc exhibited 3 log reduction of bacterial population after 12 h incubation. The treatments with 0.2% clove extract + 2% glycine, as well as treatments with 0.1% clove extract + 2% glycine + 2% NaOAc decreased the population of *E. coli* by approximately 3.0 log units after incubated 24 h, while the number of bacteria in individual factors treatment increased after 48 h incubation.

For PBS studied, incubation at 15 °C, individual 2% glycine or NaOAc was unable to suppress the tested bacteria. In contrast, the combination of clove extract with glycine and NaOAc showed the highest efficacy against *E. coli* O157:H7 and completely reduced bacteria after 96 h. Besides, the combination of 0.2% clove extract with 2% glycine or NaOAc showed strong antimicrobial activity compared to individual factors used under neutral and mildly acidic conditions. After incubation at 35 °C under neutral condition, adding an individual of 0.1% clove extract or 2% glycine exhibited about 3 log reduction, while adding 2% NaOAc and control sample did not change bacterial population after 96 h. The combinations of 0.1% clove + 2% glycine + 2% NaOAc showed the highest reduction of *E. coli* O157:H7, which at population size 3.7 logs after 24 h and complete inhibition after 48 h. For weakly acidic conditions, individual treatment of 0.1% clove extract, 2% glycine, or 2% NaOAc slowly decreased the *E. coli* O157:H7 count of about 2 log units after 96 h. However, the combination of 0.1% clove with 2% NaOAc significantly reduced the number of *E. coli* O157:H7 by about 5 log units after 24 h. Based on the results of this study, the survival of *E. coli* O157:H7 incubated at 15 °C was required higher plant extracts concentration than incubated at 35 °C, regardless of pH condition. The reducing storage temperature reduced the inhibitory effect of all plant extracts tested, indicating that the application of the tested plant extracts may protect in the case of storage at a lower temperature.

In the last part of this study, thermal processing is the most widely used to eliminate microorganisms in the juice product. However, heat treatment can damage the nutrition and

sensory quality of the juice. It is interesting to combine mild heat with natural antimicrobials in juice products. Thus, the objectives of this study were to investigate the efficacy of ethanolic clove extract against pathogenic *E. coli* O157:H7 in different culture treatments (NB, PBS, and tomato juice) at 35°C. Also, this study investigated the efficacy of ethanolic clove extract against pathogenic *E. coli* O157:H7 in tomato juice at refrigeration temperature (4°C) and room temperature (15°C). Furthermore, this study was conducted to evaluate the inactivation of *E. coli* O157:H7 by the combination of clove extract with mild heat treatment in PBS and tomato juice.

The results showed that the *E. coli* O157:H7 was the most sensitive to ethanolic clove extract in PBS, followed by tomato juice and NB, respectively. Tomato juice stored at 15 °C, clove extract exhibited higher inhibitory activity compared with at 4 °C. The combined effect of clove extract with mild heat reduced the concentration of clove extract, temperature severity, or heating period, resulting in the prevention of thermal damage to the juice. The combined effect of 0.05% clove extract with mild heat treatment at 60°C for 30 minutes achieved 5 log reduction of *E. coli* O157:H7. Besides, tomato juice with 0.05% clove extract was acceptable in all attributes in the sensory test. This finding could have been a good potential for improving the process of juice production.