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Highly enhanced bactericidal effects of medium chain fatty acids (caprylic, capric, and lauric acid) combined with edible plant essential oils (carvacrol, eugenol, β-resorcylic acid, *trans*-cinnamaldehyde, thymol, and vanillin) against *Escherichia coli* O157:H7

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thymol (PubChem CID: 6989)
vanillin (PubChem CID: 1183)

ABSTRACT

Medium chain fatty acids (MCFAs) and essential oils (EOs) are known to be natural antimicrobials, but their combined effects have not been fully investigated. The objective of the present study was to examine the bactericidal effects of various combined treatments of MCFAs [caprylic (CA), capric (CPA), and lauric acid (LRA)] and EOs [carvacrol (CAR), eugenol (EUG), β-resorcylic acid (RA), *trans*-cinnamaldehyde (TC), thymol (TM), and vanillin (VNL)]. *Escherichia coli* O157:H7, was treated with 1) control (2% ethanol), 2) MCFA alone, 3) EO alone, and 4) different combinations of MCFAs and EOs at 37 °C for 5 and 10 min. Synergistic bactericidal effects were observed with combined treatments; the log reduction in viable bacteria in response to the combined treatments was much greater than the sum of the effects of the two compounds applied individually. For example, individual treatment with 0.2 mM CPA (0.004%) and 0.4 mM RA (0.006%) for 5 min resulted in a negligible reduction in bacterial load (0.25 and 0.21 log reduction, respectively), whereas combined treatment at the same concentrations and for the same time reduced the bacterial population in the test sample to an undetectable level (initial population: 7.51 log CFU/ml; detection limit: 1 CFU/ml). The ranking of EOs showing the highest bacterial killing activities when combined with MCFAs was generally RA, CAR, TM > EUG > TC > VNL. All the antimicrobials used in this study are natural compounds that have been widely used in industry, so they are both consumer- and user-friendly. Combined treatment can overcome the disadvantages of MCFAs and EOs such as unpleasant odor and high cost because the required concentrations can be reduced. Our results indicate that the combined treatments used here could be successfully used to eliminate foodborne pathogens, significantly improving the microbiological safety of foods.

1. Introduction

Large outbreaks of foodborne pathogens continue to draw global concern about food safety for both consumers and the food industry. According to the estimation of the Centers for Disease Control and Prevention (CDC), 48 million cases of foodborne illness, 128,000 hospitalizations, and 3000 deaths were reported in the United States (Scallan et al., 2011), and the economic burden per case of foodborne illness was estimated at $1626 (Scharff, 2012). In the European Union, a total of 5648 foodborne outbreaks caused 69,553 human illnesses, 7125 hospitalizations, and 93 deaths (European Food Safety Authority, 2013). To ensure the microbiological safety of food, a wide range of technologies using physical, chemical, and biological interventions have been developed for killing pathogens in foods.

As available information about the health impacts increases, modern consumers are paying more attention to the contamination

Abbreviations: MCFA, medium chain fatty acid; CA, caprylic acid; CPA, capric acid; LRA, lauric acid; EO, essential oil; CAR, carvacrol; EUG, eugenol; RA, β-resorcylic acid; TC, *trans*-cinnamaldehyde; TM, thymol; VNL, vanillin.

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of food by pathogens as well as synthetic chemical compounds (Ibrahim, Yang, & Seo, 2008; Kim, Lee, Park, & Rhee, 2012). Consumers prefer natural compounds rather than synthetic chemicals due to concern for the perceived toxicity of chemicals; thus they have an aversion to foods treated with or containing chemical additives (Salamci, Kordali, Kotan, Cakir, & Kaya, 2007). To meet consumer criteria, the food industry is looking for naturally occurring food components with strong bactericidal effects as alternatives to chemical preservatives.

Essential oils (EOs) are oily aromatic liquids derived from plant materials including the flowers, buds, seeds, leaves, herbs, fruits, and roots of rosemary, oregano, lemongrass, sage, clove, thyme, and turmeric (Burt, 2004). They have been traditionally used as preservatives as well as flavorings and have become recognized as important natural antimicrobials (Johny, Darre, Donoghue, Donoghue, & Venkitanarayanan, 2010). Antimicrobial effects of EOs or their components are well documented against a wide spectrum of foodborne pathogens, including *Bacillus cereus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella* spp., in both bacterial suspensions and foods such as meat, dairy products, vegetables, and fruits (Burt, 2004; Hao, Brackett, & Doyle, 1998; Kim, Marshall, Cornell, JF III, & Wei, 1995; Mattson et al., 2011; Singh, Singh, Bhunia, & Stroshine, 2002; Skandamis & Nychas, 2000). Various active components of EOs obtained from plants have been identified, as follows: carvacrol in oregano, eugenol in cloves, β-resorcylic acid in angiosperms, *trans*-cinnamaldehyde in cinnamon, thymol in thyme, and vanillin in vanilla bean (Mattson et al., 2011). Despite these compounds' antibiotic activities, their application to foods is limited because high concentrations are needed to ensure food safety, and effective concentrations usually result in negative flavor and sensory changes (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Burt, 2004).

Medium chain fatty acids (MCFAs) are a family of saturated fatty acid esters of glycerol that includes caprylic acid (C8:0), capric acid (C10:0), and lauric acid (C12:0) (Zentek et al., 2011). They are naturally present in foods such as coconut oil, palm kernels, and milk of the mouse, rat, rabbit, goat, horse, elephant, and human, and are often used as nutritional supplements (Jensen, 2002; Sprong, Hulstein, & Van der Meer, 2001; Zentek et al., 2011). Antimicrobial properties of MCFAs and their corresponding monoglycerides (monocaprylin, monocaprin, and monolaurin) have been demonstrated against various foodborne pathogens such as *Campylobacter jejuni*, *Cronobacter* spp., *E. coli* O157:H7, *Helicobacter pylori*, *L. monocytogenes*, and *S. aureus* (Bergsson, Arnfinnsson, Steingrímsson, & Thormar, 2001; Bergsson, Steingrımsson, & Thormar, 2002; Desbois & Smith, 2010; Garcia et al., 2007; Jang & Rhee, 2009; Nair, Joy, & Venkitanarayanan, 2004; Thormar, Hilmarsson, & Bergsson, 2006; Wang & Johnson, 1992). Thus, MCFAs continue to attract attention as natural compounds for the control of bacteria.

A synergistic effect refers to a combination of two or more components that provide a greater effect than the sum of the effects of each single compound at the same doses (Wang, Meckling, Marcone, Kakuda, & Tsao, 2011). The main advantages of combined treatment resulting in synergistic bactericidal effects are that the loss of the nutrient content and quality of the food could be minimized by reduced quantity of antimicrobials or treatment conditions. Combined treatment could significantly save money and energy relative to the current techniques. Thus, the future of research in the area of food antimicrobials focuses on use of antimicrobials in combination with each other to result in synergistic activity (Davidson & Branen, 2005). Although MCFAs and plant EOs have been used individually to eliminate pathogens, little is known about their combined effects.

In the present study, the bactericidal effects of three MCFAs [caprylic acid (CA), capric acid (CPA), and lauric acid (LRA)] and six EOs [carvacrol (CAR), eugenol (EUG), β-resorcylic acid (RA), *trans*-cinnamaldehyde (TC), thymol (TM), and vanillin (VNL)] were investigated, either alone or in combination, to find synergistic effects with combined treatments. *E. coli* O157:H7, a major foodborne pathogen that causes serious illnesses in humans including anemia, stomach cramps, bloody diarrhea, and hemolytic uremic syndrome was selected as the test bacterium due to its strong virulence and the high mortality rate of infection (Armstrong, Hollingsworth, & Morris Jr., 1996; Faith et al., 1996; Kaper, Nataro, & Mobley, 2004).

2. Materials and methods

2.1. Test organisms and preparation of cell suspensions

The three strains of *E. coli* O157:H7 (ATCC 35150, 43889, and 43895) used in this study were obtained from the Food Microbiology Culture Collection at Korea University (Seoul, Korea). Each strain was maintained in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) containing 20% glycerol at −20 °C and subcultured at monthly intervals. Each strain of *E. coli* O157:H7 was separately cultured twice in 10 ml of TSB in screw-cap tubes at 37 °C for 24 h before use in experiments. Equal quantities of each enrichment culture were combined in a sterile plastic 50 ml centrifuge tube (Becton Dickinson, Franklin Lakes, NJ, USA) and cells were harvested by centrifugation (Centra−CL2, IEC, Needham Heights, MA, USA) at 3000 × *g* for 15 min. After the supernatant was discarded, the pellets were washed twice with 0.85% sterile saline. The final cell pellets were resuspended in 0.85% sterile saline.

2.2. Stock solution preparation of antimicrobial agents

All MCFAs and EOs were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Stock solutions of each MCFA (CA, CPA, and LRA) and EO (CAR, EUG, RA, TC, TM, and VNL) were prepared in ethanol (concentration of stock solution = 100× of the working concentration) and were used within 1 week after preparation.

2.3. Antimicrobial treatments

Experiments consisted of three parts: 1) CA + EOs, 2) CPA + EOs, and 3) LRA + EOs. Concentration of antimicrobial compounds was selected based on results of repeatedly performed preliminary experiments. The concentration which shows no bactericidal activity in individual treatments was chosen for combined treatments to examine whether they showed any synergistic effects. In the case of CA + EOs, nine treatments were done, as follows: one control treatment (2% ethanol), two individual doses of CA (0.5 and 1.0 mM), two individual doses of each EO (0.5 and 1.0 mM), and four combinations of CA + EO (0.5 + 0.5, 0.5 + 1.0, 1.0 + 0.5, and 1.0 + 1.0 mM, respectively). Experiments with LRA + EOs used the same concentrations as with CA + EOs (0, 0.5, and 1.0 mM of LRA combined with 0, 0.5, and 1.0 mM of EO). In the case of CPA + EO, because CPA has higher bactericidal activity than CA and LRA, studies with CPA + EOs used lower concentrations of both CPA and EOs (0, 0.2, and 0.4 mM of CPA combined with 0, 0.2, and 0.4 mM of EOs).

Antibacterial treatments were performed according to the previous studies (Kim & Rhee, 2015a, 2015b). In individual treatment, an aliquot (100 μl) of 20, 40, 50, and 100 mM MCFA or EO was added to 9.8 ml of sterile 0.85% saline in a sterile tube (total volume = 9.9 ml). For combined treatment, a 100 μl aliquot of both