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Short communication

Synergistic effect of carvacrol and ohmic heating for inactivation of *E. coli* O157:H7, *S.* Typhimurium, *L. monocytogenes*, and MS-2 bacteriophage in salsa

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ABSTRACT

Foodborne outbreaks have still been reported worldwide, and acid resistant foodborne pathogens are biological hazards in salsa. We investigated the combination effect of carvacrol and ohmic heating for inactivation of foodborne pathogens in salsa. Salsa samples were subjected to carvacrol, ohmic heating, and the combination treatment to identify any synergistic effect. Quality aspects of salsa such as color and lycopene content were also observed after each treatment. The synergistic bactericidal effect of combination treatment was observed for *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes*. A synergistic virucidal effect of combination treatment was also observed for MS-2 bacteriophage, which is a surrogate for human norovirus. Moreover, L* and a* values were improved by combination treatment compared to ohmic heating. Therefore, the combination treatment of carvacrol and ohmic heating could be used effectively to process salsa without incurring quality degradation.

1. Introduction

Foodborne outbreaks have still been reported worldwide even though food technology is continuously being developed to help ensure food safety. The U. S. Centers for Disease Control and Prevention (CDC) reported that one out of six people get sick, 128,000 are hospitalized, and 3000 die of foodborne illness in the United States each year (CDC, 2010). Moreover, new challenges such as demographic changes, climate changes, globalization of trade in food, and consumer's preferences for minimally processed foods have arisen (Doyle et al., 2015). Most microorganisms cannot survive at low pH an extended time, and acidic food has been considered as biologically safe (Song, Sung, & Kang, 2015). However, not only have many researchers reported that foodborne pathogens such as *E. coli* O157:H7, *L. monocytogenes*, and norovirus can survive at low pH for a long time, but also several outbreaks have been reported involving acidic foods such as juices and salsa (Lee, Kim, & Kang, 2015; Raghubeer, Dunne, Farkas, & Ting, 2000; Vimont, Fliss, & Jean, 2015). Therefore, it is necessary to ensure microbiological safety in acidic foods while maintaining quality aspects such as color, nutrient content, and flavor.

Carvacrol is a major component of certain essential oils, and possesses potential as a natural agent for food preservation by means of its antimicrobial properties (Helander et al., 1998; Lambert, Skandamis, Coote, & Nychas, 2001; Solórzano-Santos & Miranda-Novales, 2012). The possible antimicrobial mechanism of carvacrol is to increase permeability of the cytoplasmic membrane or cause oxidative damage to DNA in bacteria, whereas it targets capsids and subsequent RNA in viruses (Chueca, Pagán, & García-Gonzalo, 2014; Gilling, Kitajima, Torrey, & Bright, 2014; Ultee, Kets, & Smid, 1999). Resistance of microorganisms increase in the food matrix, and thus a high concentration of carvacrol is needed to ensure microbiological safety in food. Because such a high concentration of carvacrol results in undesirable flavor, the concentration should be reduced. Several research have recently reported

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that combining carvacrol with other treatments such as medium chain fatty acids, mild heat, and pulsed electric fields could reduce the concentration of carvacrol needed (Ait-Ouazzou, Espina, García-Gonzalo, & Pagán, 2013; Kim & Rhee, 2016).

Ohmic heating facilitates uniform and rapid heating inside of food by means of electric current through food components (Ramaswamy, Marcotte, Sastry, & Abdelrahim, 2014). Quality aspects of food could be improved by ohmic heating compared to conventional heating (Leizerson & Shimoni, 2005). In particular, solids and liquids are heated simultaneously with ohmic heating, and solid-liquid food such as salsa could be processed effectively (Lee, Ryu, & Kang, 2013). Nevertheless, there still exist limitations. First, the heating rates of solids and liquids would differ significantly if the electrical conductivity of solids and liquids are different (Ye, Ruan, Chen, & Doona, 2004). Secondly, quality aspects of solid-liquid food could be degraded due to severe heat damage. To overcome these limitations, combination treatments of ohmic heating with other technologies or chemicals have been investigated recently (Choi, Lee, Kim, & Jun 2015; Moreno et al., 2016). However, to the best of our knowledge, the combination treatment of carvacrol and ohmic heating has not been reported.

In the present study, we investigated the combination effect of carvacrol and ohmic heating in salsa. First, a synergistic bactericidal effect of combination treatment was identified for *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*. Secondly, we identified a synergistic virucidal effect of combination treatment for MS-2 bacteriophage. Finally, quality aspects including color and lycopene content were compared between ohmic and combination treated salsa. Even though widespread outbreaks caused by human norovirus have been reported worldwide, it is still not possible to cultivate human norovirus in vitro (Moore, Goulter, & Jaykus, 2015). Feline calicivirus (FCV), murine norovirus (MNV), and MS-2 bacteriophage are used as representative human enteric virus surrogates. MS-2 bacteriophage was used as a surrogate for human norovirus in the present study.

2. Materials and methods

2.1. Bacterial cultures and cell suspension

Three strains each of *E. coli* O157:H7 (ATCC 35150 (American Type Culture Collection, Rockville, MD), ATCC 43889, ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 43971, DT 104), and *L. monocytogenes* (ATCC 19111, ATCC 19115, ATCC 15313) were obtained from the bacteria culture collection of Seoul National University (Seoul, Korea). Stock and working cultures were prepared according to a previously described method (Kim & Kang, 2015a). A single colony cultivated from frozen stocks on tryptic soy agar (TSA; Difco, Becton, Dickinson, Sparks, MD) was inoculated into 5 ml of tryptic soy broth (TSB; Difco, Becton, Dickinson, Sparks, MD), incubated at 37 °C for 24 h, collected by centrifugation at 4000 × g for 20 min at 4 °C, and washed three times with 0.2% peptone water (PW; Bacto, Becton, Dickinson, Sparks, MD). The final pellets were resuspended in 0.2% PW, corresponding to approximately 10^8–10^9 CFU/ml. Afterwards, suspended pellets of the three pathogens were combined to comprise a mixed culture cocktail containing approximately equal numbers of cells of each strain of *E. coli* O157:H7 (10^7 CFU/ml), *S.* Typhimurium (10^7 CFU/ml), and *L. monocytogenes* (10^6 CFU/ml).

2.2. Sample preparation and inoculation

Pasteurized salsa (pH 3.7) was purchased at a local grocery store (Seoul, South Korea). The salsa contained no chemical preservatives and included tomatoes, jalapeño peppers, onions, garlic, and distilled vinegar. Twenty five g of salsa were put into the ohmic heating chamber at room temperature (22 ± 1 °C). A mixed culture cocktail (0.2 ml) was inoculated into 25 g of each prepared salsa sample before treatment.

2.3. Carvacrol, ohmic heating, and combination treatment

Inoculated salsa samples were treated with carvacrol, ohmic heating, and carvacrol + ohmic (combination) treatment. Purified carvacrol (98%) was purchased from Sigma-Aldrich (St. Louis, MO). Final concentration of carvacrol in salsa was adjusted to 1.3 mM (0.2 mg/g) which showed less than 0.2 log reduction for *E. coli* O157:H7 (Ait-Ouazzou et al., 2013). Ohmic heating was carried out in a previously described apparatus (Kim & Kang, 2015b). Salsa without or with carvacrol was subjected to pulsed ohmic heating (0.05 duty ratio, 60 Hz) with fixed electric field strength of 12.1 V_{rms}/cm. Addition of carvacrol did not significantly ($p > 0.05$) influence the temperature history (Fig. 1). Samples were taken at regular intervals, and populations of surviving microorganisms were enumerated.

2.4. Bacterial enumeration

For microbial enumeration, each treated 25 g sample was immediately transferred into a sterile stomacher bag (Labplas, Inc., Sainte-Julie, Quebec, Canada) containing 225 ml of sterile 0.2% peptone water and homogenized for 2 min using a stomacher (Easy Mix; AES Chemunex, Rennes, France). After homogenization, 1 ml samples were 10-fold serially diluted with 9 ml of sterile 0.2% peptone water and 0.1 ml of stomached or diluted samples were spread plated onto each selective or non-selective medium. Sorbitol MacConkey (SMAC) agar (Difco), xylose lysine deoxycholate (XLD) agar (Difco), and Oxford agar base (OAB; Difco) with antimicrobial supplement (Bacto Oxford antimicrobial supplement; Difco) were used as selective media for enumeration of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. All plates were incubated at 37 °C for 24–48 h before counting colonies characteristic of the pathogens. The overlay method (OV), developed by (Hartman, Hartman, & Lanz, 1975), and verified by (Lee & Kang, 2001) was used to recover sub-lethally injured cells of *S.* Typhimurium and *L. monocytogenes*. After cells were resuscitated on tryptic soy agar (TSA; Difco) at 37 °C for 2 h, plates were overlaid with 7–8 ml of XLD and OAB for *S.* Typhimurium (XLD-OV) and

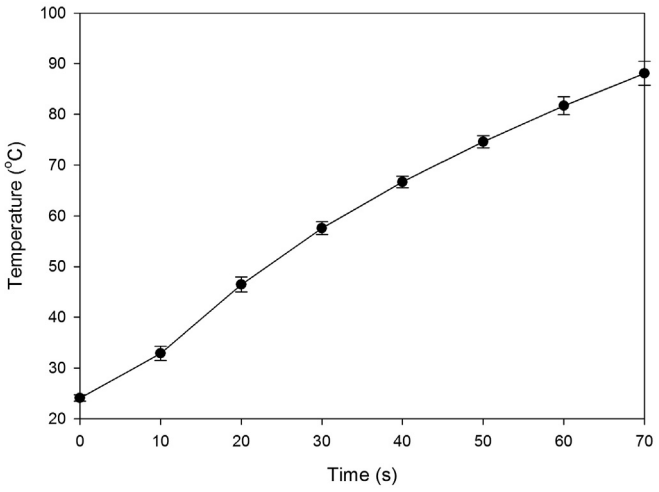

Fig. 1. Temperature history of salsa subjected to ohmic heating without carvacrol. The temperature history was not significantly influenced by addition of 1.3 mM carvacrol.