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Combination treatment of ohmic heating with various essential oil components for inactivation of food-borne pathogens in buffered peptone water and salsa

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ABSTRACT

Consumer preference for minimally processed foods has steadily increased for several years, while foodborne outbreaks from under-processed foods continue to be reported worldwide. We investigated the combination effect of ohmic heating with various essential oil components for inactivation of foodborne pathogens in buffered peptone water and salsa. We choose carvone, eugenol, thymol, and citral to combine with ohmic heating, which are registered for use as flavorings in foodstuffs. Combination treatment of ohmic heating with citral showed the most synergistic bactericidal effect against *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in buffered peptone water followed by thymol, eugenol, and carvone. When enumerated on selective media, the reductions were 4.8, 5.7, and 4.3 log CFU/ml for *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. Cell membrane destruction by combination treatment and the loss of cell membrane potential by essential oil components were proposed as the bactericidal mechanism. When applied in salsa, inactivation of bacterial pathogens was the greatest with the ohmic and thymol combination treatment followed by citral, eugenol, and carvone. A synergistic virucidal effect was observed for MS -2 bacteriophage, which was used as a norovirus surrogate. Color (b* values) of salsa were improved by combination treatment of ohmic heating and thymol compared to ohmic treated samples. Therefore, the combination treatment of ohmic heating and thymol could be used effectively to pasteurize salsa.

1. Introduction

Salsa is a piquant Mexican sauce, which usually consists of multiple ingredients such as fresh tomatoes, jalapeño peppers, onions, coriander leaves, and seasonings, and has become popular throughout the world (Sung & Kang, 2014). Acidic/acidified food products such as salsa and juice have been historically regarded as safe, but several foodborne illness outbreaks have been reported associated with low pH food products (Franco, Hsu, & Simonne, 2010; Lee, Kim, & Kang, 2015). Salsa was implicated in 70 foodborne outbreaks which resulted in 2280 illness cases from 1990 to 2006, and *Salmonella*, *Campylobacter jejuni*, *Shigella*, *Staphylococcus aureus* and norovirus were considered to be the major causal agents (Franco & Simonne, 2009). Moreover, a multi-state outbreak of *Salmonella* Saintpaul associated with jalapeño and serrano peppers was reported in the United States in 2008 (Centers for Disease Control and Prevention, 2008). Considering the increasing popularity of Mexican restaurants in the United States, Mexican dishes that incorporate jalapeño and serrano peppers, such as salsa and guacamole, are potential vehicles of foodborne illness (Neetoo & Chen, 2012).

Consumer preference for natural, healthful and minimally processed foods has steadily increased for several years. Meanwhile, new challenges in food safety such as demographic changes, climate changes, and globalization of trade have arisen (Doyle et al., 2015). Moreover, foodborne outbreaks are still being reported worldwide. The U. S. Centers for Disease Control and Prevention (CDC) reported that 3000 people die because of foodborne outbreaks in the United States each year (CDC, 2010). Even though it is crucial to inactivate foodborne pathogens to ensure the

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microbiological safety of food, the quality of food may degrade during processing. Therefore, combining several mild preservation techniques simultaneously, namely hurdle technology, has been used to satisfy both microbiological safety and quality concerns of foods (Karatzas, Bennik, Smid, & Kets, 2000). Several recent research investigations have reported that hurdle technology can be used effectively as an alternative to individual treatments (Ha & Kang, 2015; Park & Kang, 2015; Sagong et al., 2013; Sung, Song, Kim, Ryu, & Kang, 2014).

Thermal processing is still widely used in the food industry (Pereira & Vicente, 2010). However, heat transfer is accomplished by conduction and convection in many cases, with an accompanying cold spot present inside of food (Kim & Kang, 2015b). To overcome this limitation, ohmic heating (OH) has been proposed as an alternative technology. OH facilitates rapid and uniform heating inside of food by means of electric current passing through the food component (Ramaswamy, Marcotte, Sastry, & Abdelrahim, 2014). Quality aspects of food could be improved by ohmic heating compared to conventional heating. In particular, solids and liquids are heated simultaneously with ohmic heating, and solid-liquid foods such as salsa could be processed effectively. Therefore, we chose ohmic heating as the thermal process. Even though heating uniformity of ohmic heated samples is better than that of conventionally heated samples, quality can be degraded due to severe heat damage (Kim et al., 2016). To decrease the heating temperature and treatment time, combination treatments of OH with other technologies have been investigated recently (Choi, Lee, Kim, & Jun 2015; Moreno et al., 2016).

Essential oil components have potential as a natural agent for food preservation by means of their antimicrobial properties (Lambert, Skandamis, Coote, & Nychas, 2001; Solórzano-Santos & Miranda-Novales, 2012). Because resistance of microorganisms increases in food matrices, a high concentration of essential oil components is required to ensure microbiological safety. However, such a high concentration of essential oil components results in undesirable flavor. Therefore, combination treatments of these components with other technologies have been reported to reduce the concentration of essential oil components needed (Friedman, Zhu, Feinstein, & Ravishankar, 2009; Karatzas et al., 2000; S.; Kim & Rhee, 2016). There are many essential oil components which are known to have antimicrobial properties such as carvone, carvacrol, cinnamaldehyde, citral, decanal, eugenol, and thymol (Di Pasqua, Hoskins, Betts, & Mauriello, 2006). Among them, we chose carvone, eugenol, thymol, and citral to combine with OH, since they are registered for use as flavorings in foodstuffs by the European Commission (Di Pasqua et al., 2006). To the best of our knowledge, the combination treatment of these components with OH has not been reported.

In the present study, we compared the combination treatment of OH with carvone, eugenol, thymol, and citral for inactivation of *Escherichia coli* O157:H7, *S.* Typhmurium, *L. monocytogenes*, and MS-2 bacteriophage. First, the bactericidal and virucidal effect of combination treatment was compared in buffered peptone water (BPW) and salsa. Secondly, we identified a bactericidal mechanism of combination treatment using the PI and $DiBAC_4(3)$ uptake tests. Finally, quality aspects including color and lycopene content were compared. 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, South 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. 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 (10^9 CFU/ml), *S.* Typhimurium (10^9 CFU/ml), and *L. monocytogenes* (10^8 CFU/ml). A cocktail consisting of 3 microorganisms can be used in this type of experiment. This approach has precedent, since this type of mixed species culture cocktail was used in the several research-investigations (Al-Holy & Rasco, 2015; Park & Kang, 2013).

2.2. Sample preparation and inoculation

Sterile buffered peptone water (BPW; Difco, Sparks, MD, pH 7.2) and pasteurized salsa (pH 3.7) were used in this experiment. Each sample was stored in a refrigerator (4 °C) and removed 1 h prior to inoculation to equilibrate to room temperature (22 ± 1 °C). Pasteurized salsa was purchased at a local grocery store (Seoul, South Korea), and contained no chemical preservatives and included tomatoes, jalapeño peppers, onions, garlic, and distilled vinegar. Fifty ml (BPW) or g (salsa) of each sample were put into the OH chamber. A mixed culture cocktail (0.2 ml) was inoculated into each prepared sample before treatment. The final bacterial populations were 10^6–10^7 CFU/g for *E. coli* O157:H7 and *S.* Typhimurium and 10^5–10^6 CFU/g for *L. monocytogenes*.

2.3. Essential oil components preparation

Carvone, eugenol, thymol, and citral were purchased from Sigma-Aldrich (St. Louis, MO). Each essential oil component was mixed with 99.5% ethanol (concentration of stock solution = 100 × of the working concentration) and used within 1 week after preparation (S. Kim & Rhee, 2016). The ethanol concentration in the final product was 0.99%.

2.4. Bactericidal treatments

Inoculated samples were treated with individual essential oil components, OH, or essential oil components + OH (combination treatment). Concentration of essential oil components (1 mM) and treatment time were selected based on preliminary experiments. The treatment times were 60 s and 38 s for BPW and salsa, respectively. The OH system (Fig. 1A) consisted of a function generator (catalog number 33210A; Agilent Technologies, Palo Alto, CA), a precision power amplifier (catalog number 4510; NF Corp., Yokohama, Japan), a two-channel digital-storage oscilloscope (catalog number TDS2001C; Tektronix, Inc., Beaverton, CO), a data logger (catalog number 34970A; Agilent Technologies), and an ohmic heating chamber (Fig. 1B). The function generator produced various waveforms at frequencies from 1 mHz to 10 MHz and a maximum output level of 5 V. The signals generated through the power amplifier were amplified up to a maximum output of 141 V alternating current (AC). The signals expanded by the power amplifier were delivered to each of two titanium electrodes. The two-channel digital storage oscilloscope was used to measure