LWT - Food Science and Technology 84 (2017) 91–98

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Synergistic effects of combined ultrasound and peroxyacetic acid treatments against *Cronobacter sakazakii* biofilms on fresh cucumber

Hyeon-Jo Bang, Shin Young Park, Seh Eun Kim, Mizan Md Furkanur Rahaman, Sang-Do Ha*

Department of Food Science and Technology, Advanced Food Safety Research Group, BrainKorea21 Plus, Chung-Ang University, 72-1 Nae-Ri, Ansung, Kyunggido 17546, Republic of Korea

ARTICLE INFO

Article history:
Received 15 November 2016
Received in revised form
5 April 2017
Accepted 21 May 2017
Available online 29 May 2017

Keywords:
Cronobacter sakazakii biofilms
Ultrasound
Peroxyacetic acid
Synergistic effects
Cucumber

ABSTRACT

This study investigated the synergistic effects of combined ultrasound (US; 37 kHz, 380 W for 10–60 min) and peroxyacetic acid (PAA; 50–200 ppm) on reducing *Cronobacter sakazakii* biofilms on cucumbers. US was not sufficient to eliminate *C. sakazakii* biofilms (0.04–0.60 log-reduction), whereas PAA (200 ppm) significantly ($p < 0.05$) reduced biofilm formation on cucumber (0.89–1.88 log reduction). Furthermore, the combination of 60 min US and 200 ppm PAA resulted in an additional 1.63 log-reduction of *C. sakazakii* biofilms (3.51 log-reduction = 1.88 + 1.63 log). Synergistic reduction of *C. sakazakii* biofilms was observed in most combined treatment, although the most synergistic reduction values were <1.0 $\log_{10}$ CFU/cm^2. The highest synergistic value of reduction in cucumber was 1.03–1.08 $\log_{10}$ CFU/cm^2 when treated with a combination of 60 min US and 150–200 ppm PAA. In addition, the Hunter color("L", "a", and "b"), moisture contents(%), and texture(hardness and chewiness) after combined treatment with 60 min US and 200 ppm PAA did not differ significantly from those of cucumbers exposed to a single treatment. These results indicate that combined treatment with 60 min US and 150–200 ppm PAA could be a useful approach to reduce *C. sakazakii* biofilms on fresh-produce and could help enhance their shelf-life during transportation and storage.

1. Introduction

The International Commission for Microbiological Specifications for Foods (ICMSF) has ranked *Cronobacter sakazakii* as a 'severe hazard for restricted populations, life threatening or substantial chronic sequelae over a long duration' (ICMSF, 2002). Accordingly, *C. sakazakii* is considered an emerging opportunistic food-borne pathogen. The outbreaks of *C. sakazakii* infection have been documented with infant formulas, but the well-known presence of this emerging pathogen from a wide range of ready-to-eat foods, including fresh-cut cucumber and other raw vegetables, makes it highlighted to draw more attention about its role in the environment (Kim & Beuchat, 2005). It has increasingly gained the interest and concern of regulatory agencies, the scientific community, and the food industry because of its potential impact on human health (Chang, Chiang, & Chou, 2009). The infections caused by this species have predominantly involved neonates and infants less than one year of age (FAO/WHO, 2004). However, *C. sakazakii* is also linked to life-threatening infections in elderly immnocompromised adults (Park, Mizan, & Ha, 2016). Therefore, *C. sakazakii* is of public health significance for neonates and adults.

C. sakazakii contamination occurs widely in various foods such as, fresh vegetables, fruits, grains, milk, cheese, meat, and fish (Gurtler, Kornacki, & Beuchat, 2005). Especially, *C. sakazakii* has been commonly isolated from fresh produce. Because of its presence in the environment, there is a risk of contamination of fresh produce with *C. sakazakii*. Its ability to grow at temperatures as low as 5.5 °C (Nazarowec-White and Farber, 1997) raises concern about survival and growth on fresh-cut produce when refrigerated in retail markets and at home.

Attachment of bacterial cells to surfaces may be followed by bacterial growth, production of exopolysaccharides (EPS), and biofilm formation (Kumar & Anand, 1998). *C. sakazakii* has been reported to attach and form biofilms on diverse food contact surfaces such as silicone, latex, polycarbonate, stainless steel, glass

* Corresponding author. School of Food Science and Technology, Chung-Ang University, 72-1 Nae-ri, Daeduk-myun, Ansung, Gyunggido 17546, Republic of Korea.
E-mail address: sangdoha@cau.ac.kr (S.-D. Ha).

http://dx.doi.org/10.1016/j.lwt.2017.05.037
0023-6438/

(Iversen, Lane, & Forsythe, 2004; Lehner et al., 2005) as long as food surfaces, including fresh produce (Park et al., 2016). It is now recognized that biofilms are a frequent source for infections (Costerton, Stewart, & Greenberg, 1999). Approximately 80% of persistent bacterial infections in the United States were found to be associated with biofilms (Janssens et al., 2008). The survival and growth characteristics of *C. sakazakii* was tested on juices prepared from several fruits and vegetables and found that *C. sakazakii* were decreased in vegetable juices but increased in juices of different fruits (Kim & Beuchat, 2005). Beuchat et al. (2009) investigated that *C. sakazakii* grows well in fresh-cut produce at 25 °C. Therefore, fresh produce represents potential vehicles for *C. sakazakii* infections in infants and immnocompromised adults.

Compared with previously reported *Cronobacter*-contaminated foods and its biofilms, *Cronobacter* spp. are potential food-borne pathogens, and various studies are being conceded towards developing improved approaches for its inactivation (Joshi, Howell, & D'Souza, 2014). There is an urgent need to further investigate the effects of diverse physical and chemical disinfectant techniques and combination treatments to inactivate *C. sakazakii* in diverse produce, especially fresh produce.

Ultrasound (US) is a well-known washing technique used in various food industries. It is also used as an efficient biofilm removal method (Oulahal-Lagsir, Martial-Gros, Boistier, Blum, & Bonneau, 2000). However, it has been documented that even though lower-frequency in sonation is remarkably more efficient than higher frequency in for reducing biofilm cells viability, biofilms in food industries cannot be solely eliminated using the present ultrasonic technologies. Therefore, combining US techniques with other treatments are recommended (Piyasena, Mohareb, & McKellar, 2003). Due to this reason, US has been used in combination with aqueous sanitizers such as chlorine and chlorine dioxide, showing better results (Cao et al., 2010). Sodium hypochlorite, chlorine dioxide, and peracetic acid-based sanitizers as the chemical treatments can be used to reduce populations of microorganisms on fresh vegetables and fruits (Kim, Ryu, & Beuchat, 2006). Although chlorine is the most commonly used sanitizer, some studies have shown that chlorine can cause sensory quality deterioration, unpleasant odor, residual chlorine, and formation of chlorinated disinfection by-products such as trihalomethanes (THMs) with potential adverse health effects, similar to carcinogenic substances, at high concentrations (Kim et al., 2008; Parish et al., 2003; Park et al., 2016; Rico, Martin-Diana, Barat, & Barry-Ryan, 2007). Therefore, there is a need to identify alternatives for disinfection. Among commercial alternatives to chlorine, peroxyacetic acid (PAA), has been shown to reduce food-borne pathogens in fresh vegetables process wash water without the persistent toxic or mutagenic residuals or by-products and with lower dependence on pH than chlorine (Kitis, 2004; Lopez-Galvez et al., 2012). PAA is a combination of peracetic acid (CH_3CO_3H) and hydrogen peroxide (H_2O_2), usually commercialized as a liquid. It is a strong oxidant agent and is used to wash fruits and vegetables. PAA is effective at the inactivation of pathogenic bacteria in suspension at lower concentrations than the ones required when using chlorine (Artés, Gómez, Aguayo, Escalona, & Artés-Hernández, 2009; Sapers, 2003). Furthermore, it cannot be deactivated by catalase and peroxidase-enzymes that degrade H_2O_2. This agent also decomposes into safe and environmentally friendly residues in food (S. Srey, Jahid, & Ha, 2013).

Hurdle technology describes a combination of two or more different control techniques that have been proven to be effective. However, in order to achieve effective treatment, the right combination is needed. The efficacy of the combined treatments is usually dependent on the types of treatment, frequency of US, exposure time, and concentration of sanitizer (Singla, Ganguli, & Ghosh, 2011). The main goal is to use combined treatments that do not have detrimental effects on the quality attributes of the produce (Parish et al., 2003).

Therefore, there is a need to further examine the reduction and potential synergistic effects of combination treatments using US on food decontamination, especially during the washing process. The present study therefore aimed to investigate the synergistic effects of US (37 kHz, 380 W for 10–60 min) and PAA (50–200 ppm) on *C. sakazakii* biofilm reduction in fresh cucumber, compared to US or PAA alone, and its effects on the quality of the cucumber.

2. Materials and methods

2.1. Bacteria mix preparation

C. sakazakii ATCC 12868 (isolated from human), ATCC 29004 (clinical specimens), and ATCC 29544 (isolated from humans) were used as a cocktail in this study. The bacteria were recovered from −80 °C frozen stock, and 100 μL of the stock was inoculated into 10 mL of tryptic soy broth (TSB, Becton, Dickinson and Company, Detroit, MI, USA). Then, the suspension was incubated at 30 °C for 24 h in a shaking incubator (Vision Scientific, VS-8480, South Korea) at 220 rpm. Subsequently, the *C. sakazakii* cultures were centrifuged at 11,000 × g for 10 min, washed, and resuspended in fresh TSB to a final OD600 of 1.0. Then, three strains were prepared in a 1:1:1 ratio and the bacteria population in the resulting suspension were confirmed to be 10^6–10^7 CFU/mL.

2.2. Biofilm formation

Biofilms were formed in a microtiter plate following the previously described method by Stepanović, Cirković, Ranin, & Svabić-Vlahović., (2004), Stepanović et al. (2007), and Jahid, Han, Srey, and Ha (2014) with some modifications. Twenty microliters of the bacterial mix was inoculated into each well of the 96-well flat-bottom microtiter plate (Becton Dickinson Falcon, Cockeysville, MD) followed by 230 μL of sterile TSB. The plate was sealed and statically incubated at 37 °C for 48 h. According to a preliminary experiment, which compared the biofilm formation strength of different incubation temperatures and durations (data not shown), the strains used in this study made strong biofilm formation at 37 °C with 48-h incubation. Therefore, this condition was used in the biofilm formation for the disinfection test. Sterile TSB (250 μL) was used as a negative control for the biofilm-forming index (BFI) assay. BFI was calculated as previously described (Jahid, Lee, Kim, & Ha, 2013).

2.3. Disinfection assay by BFI

After 48-h incubation, the optical density (OD) of the total bacteria in the microtiter plate was measured at a wavelength of 600 nm on a microtiter plate reader (Spectra Max 190, Molecular Devices, Sunnyvale, CA). The planktonic cells and medium were removed, and each well was rinsed three times with 250 μL of sterile phosphate-buffered saline (PBS, pH 7.2) to remove the loosely attached cells. Then, US and PPA were treated individually or it's combination; instead of disinfectant, PBS was used for treating positive control (a well with *C. sakazakii* biofilm not subjected to disinfectant challenge) and negative control (well with TSB without *C. sakazakii* biofilm). The disinfectant was discarded by pipetting and 250 μL of Dey/Engley (D/E) neutralizing broth (Difco) was introduced into each well for 5 min, including the control wells. Finally, the wells were rinsed three times with 250 μL PBS. The biofilms were fixed with 250 μL of extra pure methyl alcohol (Daejung Chemicals & Metals Co., Ltd., Shiheung, Korea) for 15 min.