Food Control 73 (2017) 889–899

Contents lists available at ScienceDirect

Food Control

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

Efficacy of low concentration neutralised electrolysed water and ultrasound combination for inactivating *Escherichia coli* ATCC 25922, *Pichia pastoris* GS115 and *Aureobasidium pullulans* 2012 on stainless steel coupons

Lin Zhao [a, b], Ying Zhang [a, c], Hongshun Yang [a, b, *]

[a] Food Science and Technology Programme, Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore, 117543, Singapore
[b] National University of Singapore (Suzhou) Research Institute, 377 Lin Quan Street, Suzhou Industrial Park, Suzhou, Jiangsu, 215123, PR China
[c] Shenzhen Timevalue Information Technology Co., Ltd., 9 Keji Road, Shenzhen, Guangdong, 518055, PR China

ARTICLE INFO

Article history:
Received 29 July 2016
Received in revised form
27 September 2016
Accepted 28 September 2016
Available online 29 September 2016

Keywords:
Electrolyzed water
Ultrasound
Air-dried cells
Coupon
Atomic force microscopy (AFM)
Sanitization
Protein leakage
Mechanism
Escherichia coli
Bacteria
Yeasts
Moulds
Stainless steel
Food safety
Organic food
Optical microscopy (OM)

ABSTRACT

The sanitising effect of low concentration neutralised electrolysed water (LCNEW, pH: 7.0, free available chlorine (FAC): 4 mg/L) combined with ultrasound (37 kHz, 80 W) on food contact surface was evaluated. Stainless steel coupon was chosen as attachment surface for *Escherichia coli* ATCC 25922, *Pichia pastoris* GS115 and *Aureobasidium pullulans* 2012, representing bacteria, yeast and mold, respectively. The results showed that although LCNEW itself could effectively reduce survival population of *E. coli* ATCC 25922, *P. pastoris* GS115 and low concentration *A. pullulans* 2012 in planktonic status, LCNEW combined with ultrasound showed more sanitising efficacy for air-dried cells on coupons, with swift drops: 2.2 and 3.1 log CFU/coupon reductions within 0.2 min for *E. coli* ATCC 25922 and *P. pastoris* GS115, respectively and 1.0 log CFU/coupon reductions within 0.1 min for *A. pullulans* 2012. Air-dried cells after treatment were studied by atomic force microscopy (AFM)/optical microscopy (OM) and protein leakage analyses further. All three strains showed visible cell damage after LCNEW and LCNEW combined with ultrasound treatment and 1.41 and 1.73 μg/mL of protein leakage were observed for *E. coli* ATCC 25922 and *P. pastoris* GS115, respectively after 3 min combination treatment, while 6.22 μg/mL of protein leakage for *A. pullulans* 2012 after 2 min combination treatment. For biofilms, LCNEW combined with ultrasound also significantly reduced the survival cells both on coupons and in suspension for all three strains. The results suggest that LCNEW combined with ultrasound is a promising approach to sanitise food equipment.

1. Introduction

Organic food has developed rapidly in recent years (Li et al., 2015; Yu & Yang, 2017). Due to its strict limitations of using pesticides and chemicals, organic food contains lower chemical contaminants than conventional counterparts. However, it is still susceptible to microbiological contamination due to using organic fertilisers (Maffei, Silveira, & Catanozi, 2013; Zhang & Yang, 2017). Therefore, proper sanitisation before consumption is a critical step to ensure organic food safety.

When many chemical sanitisers are banned or limited to be applied for organic food because of the strict regulations (NOP 5026, 2011; Zhang & Yang, 2017), electrolysed water (EW), which is produced by the electrolysis of a dilute sodium chloride solution, is gaining ever-increasing popularity in food processing due to its environmentally-friendly nature and strong sanitising effect (Hricova, Stephan, & Zweifel, 2008; Rahman, Ding, & Oh, 2010; Yang, Feirtag, & Diez-Gonzalez, 2013). Compared to acidic electrolysed water, neutralised electrolysed water (NEW) is milder and

* Corresponding author. Food Science and Technology Programme, Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore, 117543, Singapore.
E-mail address: chmynghs@nus.edu.sg (H. Yang).

http://dx.doi.org/10.1016/j.foodcont.2016.09.041
0956-7135/

safer without significantly affecting foods' nutritional values and quality. Both EW reported showing effective bactericidal activity were usually with high concentration of HClO (Afari, Hung, King, & Hu, 2016; Fang, Cannon, & Hung, 2016; Hao, Li, Wan, & Liu, 2015; Jiménez-Pichardo et al., 2016). However, the National Organic Program (NOP) of US Department of Agriculture (USDA) regulates that free available chlorine (FAC) of organic food sanitisers after processing should not be more than 4 mg/L (NOP 5026, 2011; Zhang & Yang, 2017). Therefore, for organic food, another method needs to be used together with low concentration NEW (LCNEW) to get a desirable sanitising result.

Recently, ultrasound has been applied as a sanitising practice in food processing based on its strong physical and chemical energy on microorganisms, a result of intracellular cavitation (Huang et al., 2006; São José et al., 2014). According to previous reports, combined treatment of ultrasound with other chemical sanitisers could increase the bactericidal effectiveness than each used alone (Aday & Caner, 2014; Sánchez, Elizaquível, Aznar, & Selma, 2015; Zhou, Feng, & Luo, 2009). However, the synergistic effect reported from this combination mostly focused on food itself, few studies had paid much attention to sanitising food contact surface.

As microorganisms can attach to surface in two states: air-dried and biofilms, food product has a risk of microbial contamination from the processing environment. Once microorganisms attach to the surface, they have more resistance to antimicrobial agents than their planktonic counterparts (Abdallah, Benoliel, Drider, Dhulster, & Chihib, 2014; Maifreni et al., 2015; Olszewska, Zhao, & Doyle, 2016). Whereas a number of potential mechanisms of biofilms have been proposed to explain its resistance to sanitisers, studies related to the sanitising effect and distinct mechanism on microorganisms in the air-dried adhesion state are limited (Pan, Breidt, & Kathariou, 2006; Ryu & Beuchat, 2005).

Here we investigated a new sanitising method of using LCNEW (4 mg/L FAC) with or without ultrasound to determine its sanitising effect on *E. coli* ATCC 25922, *P. pastoris* GS115 and *A. pullulans* 2012 in three states: planktonic, air-dried adhesion on stainless steel coupons, and biofilms on stainless steel coupons. For air-dried cells on coupons, the sanitising kinetics, cell morphology imaged by atomic force microscopy (AFM) and optical microscopy (OM) and the intracellular protein leakage were studied further. The objective was to assess the effect of the combined sanitising treatment in a washing operation for decontamination of stainless steel coupons, simulating food processing environment, and to develop a practical and effective sanitising process for inactivating and detaching bacteria on the organic food contact surfaces.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Escherichia coli ATCC 25922 was obtained from the Food Science and Technology Programme, National University of Singapore, originally from ATCC. *Pichia pastoris* GS115 and *Aureobasidium pullulans* 2012 were obtained from College of Food Science and Technology, Nanjing Agricultural University, China, originally isolated from food environment. For *E. coli* ATCC 25922, the stock culture was transferred to 10 mL tryptic soy broth (TSB, Oxoid, Britain) to grow for 24 h at 37 °C. For *P. pastoris* GS115 and *A. pullulans* 2012, the stock culture were transferred to 10 mL malt extract broth (MEB, Oxoid, Britain) and incubated at 30 °C and 25 °C, respectively for 48 h. After 2 consecutive transfers of all three strains, *E. coli* ATCC 25922 was incubated on tryptic soy agar (TSA, Oxoid, Britain) at 37 °C for 24 h, whereas *P. pastoris* GS115 and *A. pullulans* 2012 were incubated on potato dextrose agar (PDA, Oxoid, Britain) for 48 h at 30 °C and 25 °C, respectively. All strains were subcultured again in respective broth for use.

Bacterial density was studied by spectrophotometric analysis and plate count method following a previous lab manual (Reynolds, 2011). All working cultures (ca. 9 log CFU/mL for *E. coli* ATCC 25922, ca. 8 log CFU/mL for *P. pastoris* GS115, ca. 6 log CFU/mL for *A. pullulans* 2012) were separately centrifuged (8200 × *g*, 10 min, 20 °C) (Eppendorf, Centrifuge 5804 R, Germany) and the harvested cells were washed twice in PBS (phosphate buffer saline, pH 7.2) and resuspended for following use.

2.2. Preparation of LCNEW

The electrolysed water was generated by electrolysis device (Hoshizaki, ROX-10WB3, Hoshizaki Singapore Pte Ltd, Singapore) with a continuous supply of dilute salt solution (0.9% NaCl in deionised water). The anode solution was modified by cathode solution until pH neutral (7.0 ± 0.1), which was measured by a pH meter (Thermo Orion pH meter, Waltham, MA, USA). The FAC of 4 mg/L was obtained by dilution with sterilised distilled water and was measured by chlorine test kit (Reflectoquant Chlorine test, Chlor-Test 0.5–10.0 mg/L Cl_2, Darmstadt, Germany). The oxidation reduction potential (ORP) was measured by ORP meter (HM Digital ORP-200, Culver City, CA, USA) immediately after preparation. The ORP of the LCNEW used in this study was 750 ± 15 mV.

2.3. Preparation of the stainless steel coupons

Stainless steel coupons (1 cm in diameter and 0.7 mm in thickness, type: 430, Muzeen and Blythe Co., Winnipeg, Canada) were used as attached surface. They were prepared according to a previous method with some modifications (Kim, Ryu, & Beuchat, 2007). Before each experiment, the coupons were sonicated in 80 °C 15% (v/v) phosphoric acid solution for 20 min and rinsed with distilled water, followed by sonicating in 80 °C 15% (v/v) alkali detergent solution for 20 min and rinsed with distilled water. Finally, the coupons were sonicated in 80 °C distilled water and rinsed. After dried at 50 °C, the coupons were sterilised by autoclaving at 121 °C for 15 min.

2.4. Treatment of planktonic cells with LCNEW

The cells concentration of the inoculums were 9, 8 and 6 log CFU/mL for *E. coli* ATCC 25922, *P. pastoris* GS115 and *A. pullulans* 2012, respectively. This was the concentration of their each stationary phase as prepared in 2.1. Besides, for *A. pullulans* 2012, lower concentration (5 log CFU/mL) was also studied. Each bacterial suspension (3 mL) was mixed thoroughly with 27 mL LCNEW (sterile deionised water (DW) as control) in sterile centrifuge tubes. Suspension (1 mL) was transferred to sterile centrifuge tubes at different treatment time (0–10 min), and then 9 mL neutralising buffer (containing 5 g/L sodium thiosulfate) was added (Deza, Araujo, & Garrido, 2003). After 5 min neutralising, all samples were serially 10-fold diluted in sterile PBS and 0.1 mL were plated on TSA for *E. coli* ATCC 25922 or on PDA for *P. pastoris* GS115 and *A. pullulans* 2012, incubated in the same conditions as mentioned in 2.1. The number of the living microorganisms was determined by viable count method.

2.5. Treatment of air-dried cells on the coupons

2.5.1. Air-dried cells on the coupons

Sterile stainless steel coupons were placed in sterile petri dishes and inoculated with 0.1 mL suspensions of 9 and 8 log CFU/mL, respectively for *E. coli* ATCC 25922 and *P. pastoris* GS115 as mentioned in 2.1, to reach ca. 8 and 7 log CFU/coupon, respectively.