Food Microbiology 46 (2015) 471–478

Contents lists available at ScienceDirect

Food Microbiology

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

Cross-contamination of *Escherichia coli* O157:H7 is inhibited by electrolyzed water combined with salt under dynamic conditions of increasing organic matter

Vicente M. Gómez-López [a, b], María I. Gil [a], Laurent Pupunat [c], Ana Allende [a, *]

[a] Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS-CSIC, P. O. Box 164, Espinardo, Murcia E-30100, Spain

[b] Cátedra Alimentos para la Salud, Departamento de Tecnología de la Alimentación y Nutrición, Facultad de Ciencias de la Salud, UCAM Universidad Católica San Antonio de Murcia, Spain

[c] WaterDiam France SAS, Rue de l'école, Franken, Alsace F-68130, France

ARTICLE INFO

Article history:
Received 31 January 2014
Received in revised form
31 July 2014
Accepted 11 August 2014
Available online 16 September 2014

Keywords:
Food safety
Disinfection
Electrolysis
Process wash water
Trihalomethanes

ABSTRACT

Water can be a vector for foodborne pathogen cross-contamination during washing of vegetables if an efficient method of water disinfection is not used. Chlorination is the disinfection method most widely used, but it generates disinfection by-products such as trihalomethanes (THMs). Therefore, alternative disinfection methods are sought. In this study, a dynamic system was used to simulate the commercial conditions of a washing tank. Organic matter and the inoculum of *Escherichia coli* O157:H7 were progressively added to the wash water in the washing tank. We evaluated the effectiveness of the electrolyzed water (EW) when combining with the addition of salt (1, 0.5 and 0.15 g/L NaCl) on the pathogenic inactivation, organic matter depletion and THM generation. Results indicated that electrolysis of vegetable wash water with addition of salt (0.5 g/L NaCl) was able to eliminate *E. coli* O157:H7 population build-up and decrease COD accumulation while low levels of THMs were produced.

1. Introduction

Washing is one of the most important steps in the processing operations of fresh-cut produce. It is usually carried out in the presence of disinfectant solutions to clean vegetable pieces with unwanted residues such as soil and debris and decrease microbial load. However, washing can also serve as a vector for cross-contamination of enteric pathogens that could be eventually present. Therefore, it is important not only to use wash water with high quality but also to protect it from the continuous entrance of organic matter and microorganisms transported by the permanent incoming flow of vegetable pieces into the washing tank. A deeper discussion about problems and solutions of water disinfection for the fresh-cut industry has already been published (Gil et al., 2009).

Water chlorination is the most used disinfection method for wash water. However, the potential formation of dangerous concentrations of disinfection by-products, such as trihalomethanes (THMs), has promoted the search for other water disinfection technologies of similar or better efficacy. Electrolyzed water (EW) is produced by passing water through an electrolytic cell, where diverse disinfectant species including free chlorine are generated. An external source of chloride such as small amounts of salt (i.e. NaCl) can be added to increase the generation of free chlorine (Fallanaj et al., 2013; Gómez-López et al., 2008). Besides free chlorine, reactive oxygen species (ROS) such as $\cdot OH^-$ and O_3, have been identified as disinfection agents in EW (Jeong et al., 2006; Hao et al., 2012). EW inactivates bacteria by increasing membrane permeability and leakage of intracellular content as well as by decreasing dehydrogenase and nitrate reductase activities (Kiura et al., 2002; Zeng et al., 2010). The bactericidal effects of EW on foodborne pathogens are well documented by numerous studies (Izumi, 1999; Gómez-López et al., 2008; Park et al., 2008; Ding et al., 2010). EW has multiple advantages over the use of chlorinated water: 1) it can be produced *on-site*, 2) the raw materials (water and NaCl) are found everywhere, 3) it is environmentally friendly, 4) it reduces cost and hazards associated to chlorine handling, 5) it has low cytotoxicity and 6) the development of resistant strains has not been reported yet (Huang et al., 2008; Al-Haq and Gómez-López,

* Corresponding author. Tel.: +34 968396377; fax: +34 968396213.
E-mail address: aallende@cebas.csic.es (A. Allende).

http://dx.doi.org/10.1016/j.fm.2014.08.024
0740-0020/

2012). *Escherichia coli* O157:H7 is one of foodborne pathogens of great concern during vegetable washing as it has been associated with outbreaks in fresh-cut produce (Erickson, 2012). Electrolyzed water (EW) has been described as effective in inactivating *E. coli* O157:H7 and oxidizing the organic matter in process wash water, allowing water reuse and reducing the environmental pollution caused by water discharges (López-Gálvez et al., 2012). The electrolytic oxidation of organic pollutants has been reviewed (Anglada et al., 2009; Panizza and Cerisola, 2009). However, the increase in the chlorine formation by EW in combination with salt and the formation of THMs is not well known.

Trihalomethanes (THM) have been the main disinfection by-products of concern when chlorine is present in contact with organic matter. Two THMs, chloroform and bromodichloromethane have been classified by the WHO's International Agency for Research on Cancer as possibly carcinogenic to humans (IARC, 1999a,b). Drinking water is the classical exposure route of concern, but others routes are possible and could cause an accumulative effect in addition to drinking water, such as the dermal/inhalation exposure due to showering/bathing/swimming (Richardson et al., 2007). Huang and Batterman (2010) have described that vegetables can absorb THMs from the washing water. The generation of THMs in both process wash water and fresh-cut produce by different disinfection agents have been studied in our group (López-Gálvez et al., 2010).

Most of the studies about fresh produce washing have been performed at laboratory scale with potable water, but it has been recognized that significant differences are found when assessments are performed at a pilot or factory scale (Gil et al., 2009). Moreover, most of the studies have described bacterial inactivation in clean water and only some studies have been performed using wash water with organic matter but in almost all the cases, they were carried out in one-time-event experiment. In this regard, we have previously shown that organic matter decreases the efficacy of EW to inactivate *E. coli* O157:H7 in process water (López-Gálvez et al., 2012). To evaluate the impact of different operating factors on the efficacy of EW to inactivate *E. coli* O157:H7 and the organic matter depletion, experiments were carried out in a one-time-event. This means that free chlorine was added once the washing process has been initiated and that the washing process was simulated at a single moment with the addition of a specific COD (725 mg O_2/L) (Gómez-López et al., 2013a) or different CODs (60, 300, 500 or 750 mg O_2/L) (López-Gálvez et al., 2012). Recently Van Haute et al. (2013) published the results of a dynamic washing process for addition of chlorine but with a fixed COD value for wash water disinfection. However, in our opinion, the real situation in a washing tank, at least during the first hours, is better exemplified by a continuous addition of organic matter and microorganisms to the process water. The aim of this study was to evaluate the efficacy of EW combined with salt on *E. coli* O157:H7 inactivation in vegetable wash water simulating the process wash water from leafy vegetable processing companies. The chemical safety of the wash water during the washing process was also evaluated by measuring the organic matter depletion and THM generation.

2. Materials and methods

2.1. Bacterial strains and inoculum preparation

A five-strain cocktail of *E. coli* O157:H7 (CECT 4267, 4076, 4782, 4783, and 5947), provided by the Hibro Group from the University of Cordoba (Spain), was used in the study. Cultures were rehydrated in Brain Heart Infusion broth (BHI, Oxoid, Basingtoke, United Kingdom). Nalidixic acid-resistant (Nal^R) *E. coli* O157:H7 cultures were obtained by consecutive 24-h transfers of BHI cultures to BHI with increasing concentrations of nalidixic acid (Nal) (Merck, Darmstadt, Germany) until strains were resistant to 50 μg Nal/mL. Nal^R *E. coli* O157:H7 cultures were consecutively sub-cultured twice in 5 mL of BHI supplemented with Nal (50 μg/mL) at 37 °C for 20 h. After the second incubation, cultures were mixed, equal volumes of cell suspensions were combined to give approximately equal populations of each culture. Final concentrations of the inoculum solutions were confirmed by plating on Chromocult coliform agar (Merck, Barcelona, Spain) Nal^+ (50 μg/mL).

2.2. Wash water

The process wash water was obtained following the protocol described by López-Gálvez et al. (2010). Iceberg lettuce (*Lactuca sativa* L.) was purchased from a local wholesale market in Murcia (Spain) at the day of harvest and transported within 15 min under refrigerated conditions to the laboratory. Outer leaves were manually removed and discarded while internal leaves were cut into 3 cm pieces. Afterward, batches of 67 g of lettuce each were placed into stomacher bags (Seward Limited, London, UK). Two hundred mL of water were added and the mixture was homogenized for 2 min in a stomacher (AES Chemunex, Bruz, France). The obtained process wash water was filtered through a nylon mesh with gaps of 0.5 mm, in order to avoid obstruction of the electrolytic cell. The batch of process wash water was divided in portions and frozen at −20 °C until use. The obtained process water had a very high COD (>2000 mg/L) that needed to be diluted to attain specific COD levels. Process water was thawed overnight the day before each test, and then diluted with tap water. Thawed vegetable wash water had the following characteristics: alkalinity (150 ± 25 mg equiv. $CaCO_3$/L), turbidity (123 ± 58 NTU), and conductivity (1786 ± 126 μS/cm).

2.3. Electrochemical equipment

Disinfection experiments were performed using a bench top treatment system defined as "dynamic system" where organic matter was constantly added to the washing tank (Fig. 1A). The dynamic system included: a power supply, control board, centrifuge pump for water recirculation, two peristaltic pumps for dosing, 30 L stainless steel tank (treatment tank), flow-meter of water recirculation, two reservoir polypropylene tanks (one process water reservoir used in all experiments and one chlorine reservoir for chlorination experiments), pipes, and electrolytic cells (for electrochemical disinfection). Different configurations of the dynamic system were used in order to test different disinfection treatments. Fig. 1B shows the configuration when sodium hypochlorite was tested and Fig. 1C corresponds to the configuration for the electrochemical disinfection. The electrolytic cells were kindly provided by WaterDiam France SAS (Franken, France). Process temperature was controlled by pumping cold water through stainless steel heat exchangers immersed into the process water. The electrolysis cell DiaClean® 101 8000 with no separation between anodic and cathodic compartment was used. This cell has one boron-doped diamond (BDD) cathode and one BDD anode with an overall effective anode surface area of 67 cm^2, an electrode boron doping level (known as B/C ratio) of 8000 ppm and a current density of 60 mA/cm^2 when operated at 4 amperes (A). Amperage was set and controlled through the experiments by the power supply, which changed the polarity of electrodes every 20 min to minimize scale build-up on their surface. The vegetable wash water in the tank was pumped through the electrolytic cell and recycled back to the tank (Fig. 1C). Water recirculation rate was adjusted by means of a valve to 750 L/h. The system was assumed to be a perfectly mixed reactor; at that recirculation rate the whole volume