

Efficacy of electrolyzed water on reducing some foodborne pathogens in cutting boards

ABSTRACT

Cross-contamination is an important source of foodborne pathogens, from cutting boards, knives and food preparation surfaces. Methods for reducing or inactivating pathogens from these sources are necessary for establishing food safety. Electrolyzed water (EW), nowadays is gaining popularity as a sanitizer in the food industry. The aim of this study was to determine the efficacy of EW for reducing or eliminating *Salmonella Typhimurium*, *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* on unscarred, wooden cutting boards. For this purpose, 1 milliliter of each selected pathogen (10^9 CFU/mL) was inoculated on a different 100 cm² (10x10) unscarred cutting board. After the inoculation, the boards were air-dried for the attachment of the pathogens for 1 hour and then each cutting board immersed in 500 ml of EW (5 % concentration) at room temperature for 10 min. After that, the number of the pathogens on cutting boards and also in soaking water was determined by sampling at minutes 0 (zero) and 30, and hours 2, 8 and 24. Treatment of inoculated cutting boards with EW reduced all the pathogens populations by 1 log CFU/100 cm² at minute 0 (zero). *S. aureus* and *S. Typhimurium* was not detected after the 8th hour, also *E. coli* and *L. monocytogenes* were not survived after 2 hours. All of the pathogens were not detected in soaked EW after the treatment. This study presented that immersing cutting boards into EW could be preferred as a useful method for reducing some foodborne pathogens.

Keywords: Cleaning, decontamination, disinfection, electrolyzed water, food safety.

I NTRODUCTION

Cross contamination is commonly implicated as an important factor in the spread of foodborne diseases. Food contact surfaces, like cutting boards or utensils, have been pointed at as a route of cross-contamination, repeatedly. They could serve as a contamination source through transferring the disease agents between foods and the ones that could not be cooked further before eating, they allow bacteria to multiply particularly if raw meat or meat products remains on the surfaces (Kusumaningrum et al., 2003). Effectively sterilizing cutting boards is an important tool that minimizes the cross-contamination risk and resulting foodborne illness. However, cleaning of cutting boards with water and commercial chemical cleaners are not enough for total elimination of bacteria therefore for complete hygiene antimicrobial agents are necessary (Huang et al., 2008).

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Research Article

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Cutting boards should be cleaned and sanitized often, thus, they are used repeatedly to prepare food to a large number of people in restaurants and food producing facilities (Monnin et al., 2012). Although there are several commercial products with different properties for this purpose, research is still being conducted into the new ones. Recently in the food industry, electrolyzed water (EW) as a sanitizer, is gaining popularity by reducing or eliminating bacteria on food products, utensils, and non-food contact surfaces (Rahman et al., 2016). EW was first developed in Japan and is reported to have a strong bactericidal effect on most pathogenic bacteria important for food safety (Çil et al., 2012).

EW is produced with an electrolysis chamber which contains a separating membrane between the cathode and anode electrodes by electrolysis of NaCl solutions. By exposing the electrodes to direct current voltages, different EW solutions are generated at the negative and positive poles. Chloride and hydroxide, the negatively charged ions, in the diluted salt solution move to the anode and give up electrons and become chlorine gas, hypochlorite ion, hypochlorous acid oxygen gas and hydrochloric acid and by this reaction a solution with high ORP and a low pH is generated, called acidic electrolyzed water (AEW). Meanwhile hydrogen and sodium (positively charged ions) are moving to the cathode and taking up electrons and become sodium hydroxide and hydrogen gas. By this reaction alkaline electrolyzed water (AIEW) with a high pH and a low ORP is generated (Wang et al., 2019; Athayde et al., 2018). The presence of chlorine and a high ORP are accepted as the main antimicrobial activity of AEW. Moreover, with the development of this mechanism, a third type of electrolyzed water was produced. By using a single cell chamber without the separating membrane, Neutral EW (NEW) partially mixed with hydroxide ions, which has

an ORP of 750–900 mV a neutral pH is produced from the anode. Because of its neutral pH, in comparison with AEW, it is believed to be NEW is less corrosive for processing equipment (Ding and Liao, 2019; Subrato et al., 2012).

Safety is the main advantage of EW. EW is different from many disinfectants it is not corrosive for tissues. Because no hazardous chemicals added during the production, it has less adverse impact on the environment and it becomes ordinary water again, by the contact with organic matter or when diluted by tap water or reverse osmosis. Additionally, EW has very little side effects, is relatively cheaper than other sanitizers, it is easy to process, and microorganisms do not acquire resistance. (Al-Holy and Rasco, 2015; Xuan and Ling, 2019). The only disadvantage of this sanitizer is its effectiveness is reduced by the presence of organic matters and with time, the bactericidal activity of EW is reduced due to chlorine loss (Ogunniyi et al., 2019).

The study was designed to determine the efficacy of EW for reducing or killing *Escherichia coli*, *Salmonella* Typhimurium, *Listeria monocytogenes*, and *Staphylococcus aureus* on cutting boards and compare its stability over time.

MATERIALS and METHODS

Bacterial cultures and inoculum preparation

Common foodborne pathogens chosen for the experiment. *E. coli* NCTC 12900, *Salmonella* Typhimurium ATCC 14028, *Listeria monocytogenes* ATCC 7646 and *Staphylococcus aureus* ATCC 25923 strains were individually cultured and activated by inoculating cultures to the specific mediums. Pathogens were maintained in 10 ml of Brain Heart Infusion Broth (Oxoid CM1135) at 37°C by daily transfer. With a spectrophotometer (Thermo Scientific, TM1000) bacterial

concentrations were measured by the absorbance of the bacterial suspension at 600 nm. All strains are adjusted to a cell count of 10^9 CFU/mL before the experiment.

Electrolyzed water preparation

A commercial electrolyzed water sanitizer used for the experiment. Electrolyzed water with a concentration of 5 % were prepared in accordance with the manufacturers recommendation by using the stock. Final EW treatment solutions have characteristic odor, they are colorless, pH range of 6.9-7.0 and ORP 720-750.

Cutting board preparation and sample inoculation

To determine the antibacterial activity, cutting boards which are new (unscarred), hardwood, and 10×10 cm² size were used in the study. Each board was sterilized in the autoclave at 121 °C for 20 min (wrapped with an aluminium foil). A volume of 1 millilitre of each bacterial culture of selected pathogen (10^9 CFU/ml) was inoculated on a different cutting board and spread evenly on 100 cm² at room temperature (22-23 °C). After the inoculation, they were left to dry in a laminar flow cabinet under aseptic conditions for 1 hour at room temperature for the attachment of the strains.

Sanitization treatment

Following the attachment, each cutting board immersed in 500 ml of EW treatment solution at room temperature for 10 min. After the treatment, the surviving population of the pathogens on cutting boards and in soaking solution was determined by sampling at minutes 0 and 30, and hours 2, 8 and 24. One cutting board which is inoculated but not immersed to the EW was evaluated as a control group for

each pathogen to control the efficiency of inoculation. After immersion, by using a sterile tong each cutting board was aseptically removed and water was drained from the board (Venkitanarayanan et., 1999).

Microbiological analysis

Two sterile cotton swabs was used to swab the 100 cm² surface area of each treated cutting board and the control group boards, from right to left and from top to bottom. The swabs were transferred in a 9 ml sterile peptone water. One milliliter from this water was then serially diluted with 9 ml of sterile peptone water and 0,1 ml from each dilution plated onto specific mediums for pathogens. As specific mediums, Brilliant-Green Phenol-Red Lactose Sucrose Agar (Merck 107237) for *S. Typhimurium*, Baird Parker (Oxoid CM1127) for *S. aureus*, Oxford Agar Base (Merck, 107004) for *L. monocytogenes* and Violet Red Bile Lactose Agar (Oxoid CM0968) for *E. coli* were used. All plates were incubated at 37°C for 24-36 h and counted. Also, 1 ml of the treatment water was transferred to 9 ml sterile peptone water and enumerated likewise.

RESULTS

According to the control groups, *E. coli*, *S. Typhimurium* and *S. aureus* were attached 10^6 CFU/cm², and *L. monocytogenes* was attached 10^5 CFU/cm² on to the cutting boards. Compared to the control groups, immersion to the electrolyzed water, caused 1 log decrease in all pathogens at minute 0. *L. monocytogenes* and *E. coli* counts were determined below the detection limit at the end of the 2nd hour and *S. Typhimurium* and *S. aureus* at the end of the 8th hour (Table 1). Further, none of the pathogens were detected in the soaked water samples.

Table 1. Attachment of the control groups and reduction of pathogens after the EW treatments.

	Cutting Boards (CFU/100 cm ²)			
	<i>S. Typhimurium</i>	<i>S. aureus</i>	<i>L. monocytogenes</i>	<i>E. coli</i>
Control	10 ⁶	10 ⁶	10 ⁵	10 ⁶
23 °C 0 min	10 ⁵	10 ⁵	10 ⁴	10 ⁵
23 °C 30 min	10 ⁵	10 ⁵	10 ⁴	10 ⁵
23 °C 2 hr	10 ⁴	10 ⁵	<200	<200
23 °C 8 hr	<200	<200	<200	<200
23 °C 24 hr	<200	<200	<200	<200

DISCUSSION

Various sanitizers are used to reduce microbial loads on cutting boards surfaces; nevertheless, different factors may restrict their applications. Concentration, contact time, active ingredients, residual effect, formation of toxic by-products, effectiveness in the presence of organic matter are among these factors (Al-Qadiri et al., 2016). In our study we search the efficacy of a promising sanitizer, electrolyzed water, on to the common foodborne pathogens, on cutting boards. Our results showed that, immersing the contaminated cutting boards into the electrolyzed water easily eliminated the selected pathogens. At the end of the 8th hour, all of the pathogens were under the detection limits, when compared with the control groups. With the results of control groups, our study, also has highlighted the fact that pathogens may remain viable on cutting board surfaces and may re-contaminate other foods.

Electrolyzed water has become an effective sanitizer in food industry. In a meta-analysis of Afari and Hung (2018), developed for an overall estimation of the pathogen reductions achievement after the EW treatment of food products, results showed that when compared with the other control group sanitizers, EW was more effective in reducing pathogens on foods. The efficacy of electrolyzed water in reducing

microbial population has also been reported by other researchers. Venkitanarayanan et al. (1999) similarly indicated a higher bacterial reduction, 5 to 6 log CFU/ml, in their study. They search the effectiveness of acidic electrolyzed water on *E. coli* O157:H7 and *L. monocytogenes* on cutting board surfaces by immersion. They preferred to use smooth, plastic cutting boards and compare their results with deionized water. The reduction was determined as only 1 to 1.5 log CFU/ml in deionized water. Likewise, they enumerate the treatment waters. They reported that, no *E. coli* O157:H7 and *L. monocytogenes* were detected in EW used for the soaking cutting boards, whereas the pathogens were survived in the deionized water. In another study, it was investigated the efficacy of neutral electrolyzed water to reduce populations of *E. coli*, *Pseudomonas aeruginosa*, *S. aureus*, and *L. monocytogenes* on pine-wood cutting boards. As a result, 4 log CFU/50 cm² reduction was determined in all pathogens with soaking the cutting boards into the EW solutions for 5 minutes. In the same study, no survivors were detected in washing solutions after the treatment with NEW while, in control washing solution, an average of 6 log CFU/ml of all strains was recovered (Deza et al., 2007). Monnin et al. (2012) inoculated the cutting boards made of hardwood and bamboo with *E.*

coli K12 and *L. innocua* and washed them with neutral electrolyzed water. After the washing protocol, they found a 3 to 4 log CFU/100 cm² reduction in both microorganisms. Their findings are slightly low, when compared with previous studies results. The key difference in their methodology is, they scarred the cutting boards used in the experiment, with a serrated kitchen knife in order to simulate normal usage. It is known that, cutting boards are often scarred by knife edges over time and these scarred parts can protect bacteria from cleaning and sanitation. Same state is supported in the studies of Kusumaningrum et al. (2002) and Lee et al. (2007). Unlike, in the study of Al-Qadiri et al. (2016), they also used maple-hard wood cutting-boards scarred with a pizza-knife, to determine the reduction effect of electrolyzed water on *S. Typhimurium*, *E. coli* O157:H7, *C. jejuni*, *L. monocytogenes* and *S. aureus*. After 5 min of treatment, 4 and 5 log reductions were achieved for Gram-negative and Gram-positive strains, respectively. Fabrizio and Cutter (2003) demonstrated in their study, quite high reduction levels (> 8 log) after the EW treatment of the *S. Typhimurium* and *L. monocytogenes*. But they did their experiment on only cell suspensions. It is important to simulate the kitchen conditions in order to obtain rational results and determine the real efficacy of the disinfectant.

Another fact in different studies is the type of cutting board material used in the experiment. Plastic boards are considered easier to sanitize than wooden ones. Beside certain wood species have endogenous antibacterial properties, by their physical structure, wood is able to absorb moisture and protect bacteria from cleaning and sanitizing. Also, studies revealing that wooden boards could absorb bacterial suspension and the inner part of the wood might still remain wet and retain most of the bacteria while the surface appeared dry. Deza et al. (2007), evaluated the effectiveness of neutral electrolyzed water on *E. coli*,

Pseudomonas aeruginosa, *S. aureus*, and *L. monocytogenes*, on plastic and wooden kitchen cutting boards and reported a significant difference between the materials used. In plastic boards the reduction of bacterial populations was 5 log CFU/50 cm², whereas in wooden cutting boards it was 3 log CFU/50 cm². Monin et al. (2012) compared two cutting board material types, bamboo and hardwood, for the residual bacterial numbers on their surfaces following the sanitation with electrolyzed water. Researchers choose bamboo for the study due to its increasing popularity as a sustainable, environmentally-friendly alternative to other materials, like wood or plastic. They reported no significant differences in surviving bacteria counts between hard-wood and bamboo material. Chiu et al. (2006) studied the efficacy of EW treatment in inactivating *V. parahaemolyticus* on the surfaces of different cutting boards made from bamboo, wood and plastic). Soaking the cutting boards in EW for 5 min eliminated the *V. parahaemolyticus* on both plastic and wood boards. However, the EOW treatment resulted ineffective on the bamboo boards. Al-Qadiri et al. (2016) stated in their study, longer exposure times for NEW were required for the effectiveness against microbial contamination on wooden compared to polypropylene surfaces. In a study of Jeon et al. (2018), the reduction effect of various sanitizers on *L. innocua* biofilm cells on rubber, polypropylene, glass and stainless-steel surfaces was searched. They reported as, washing contact surfaces with 30, 50, 70, and 120 ppm of AEW reduced the population of *L. innocua* biofilm cells significantly and among the four-food contact surfaces the most effective biofilm reduction was obtained on polypropylene.

CONCLUSIONS

This study showed that, EW could be used as a chemical to sanitize the wooden cutting boards. Besides, research need to compare its effectiveness with the common disinfectants

used in the food industry. Further studies should be conducted to determine the effect of electrolyzed water on the presence of organic matter. As a result of all these studies, it will be possible to determine the most appropriate and economical use of EW

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