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Disinfection with neutral electrolyzed oxidizing water to reduce microbial load and to prevent biofilm regrowth in the processing of fresh-cut vegetables

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ABSTRACT

Product decontamination is one of the most important processes of the hygienic practice in food industries such as Minimally Processed Vegetables (MPV) plants and sodium hypochlorite (NaOCl) solutions are commonly used as a biocide for disinfection. Although it may be corrosive and irritating when compared to alternative biocides, this biocide is frequently applied at high concentrations. This work aims at studying the use of lower concentrations of chlorine by testing neutral electrolyzed oxidizing water (NEOW) as a chlorine-source disinfectant in fresh-cut salad processing. Assays were performed at industrial and laboratory scale. Results showed that lower doses of chlorine from NEOW (30 ppm) are as effective as higher concentrations of the traditional chlorine from NaOCl (80 ppm) in the reduction of total microbial population at industrial scale. Moreover, in laboratory studies, the NEOW chlorine was also more effective in biofilm eradication, as well as a biofilm preventive agent. NEOW can thus be a successful alternative water disinfection technique, reducing the free chlorine concentration needed to sanitize salads, also decreasing water consumption whilst taking into account environmental and food quality impacts.

1. Introduction

The consumption of ready-to-eat salads has increased drastically in recent years. In order to ensure the hygienic safety of a product that is consumed raw, salad washing has become a critical step in the production process (De Giusti et al., 2014). High volumes of water are frequently used to achieve microbial load reductions and, in order to maintain good hygienic practices, chlorine compounds (such as sodium hypochlorite) are frequently used as disinfectant agents. However, the free chlorine concentrations in this food industry are very high and the doses applied may fluctuate, leading to lack of knowledge regarding the water disinfection efficiency and the microbial load that remains in the process water and in the washing tanks surface. Regarding the use of hypochlorite, several concerns have been raised, namely the release of toxic chlorine by-products, accumulation of chloramines, and generation of chlorine off-gas in the processing environment (Shen et al., 2012; Vandekinderen et al., 2009) that may pose significant environmental and health risks (Ölmez and Kretzschmar, 2009).

In order to reduce water and chlorine consumption while maintaining salad-washing efficiency and avoiding the production of disinfection by-products like organochlorinated compounds, neutral electrolyzed oxidizing water (NEOW) has been proposed as a sanitizing agent. Electrolyzed oxidizing (EO) water has been regarded as a new sanitizer in recent years in the food sector, for industrial equipment and in the processing of vegetables, fruit, poultry, meat and seafood (Huang et al., 2008).

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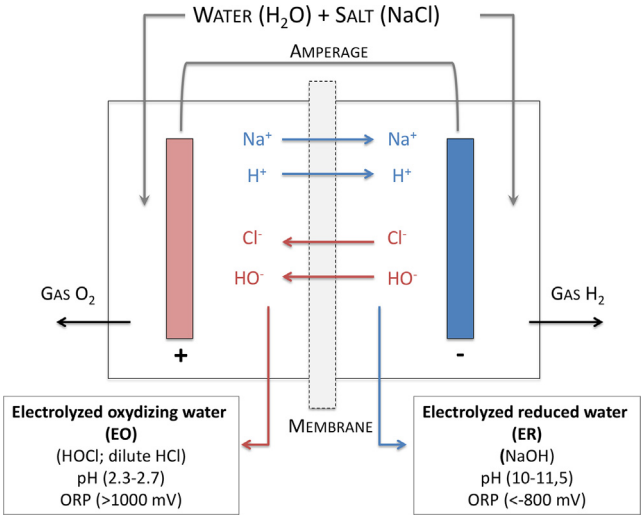

Fig. 1 – Schematic representation of electrolyzed oxidizing water production [adapted from (Huang et al., 2008)].

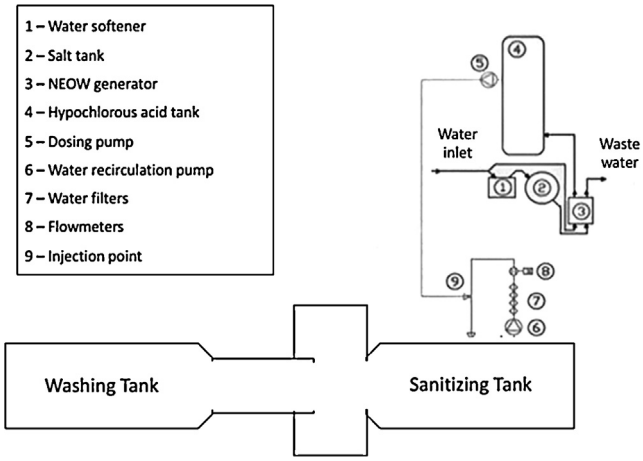

Fig. 2 – Schematic representation of the industrial washing and sanitizing tanks.

EO water is generated by electrolysis (Fig. 1) only with water and salt (sodium chloride) as raw materials, having the following advantages over other traditional cleaning agents: effective disinfection, simple operation, relatively inexpensive, environmentally friendly and safe (Huang et al., 2008). Also, the chlorine-based biocide can be produce *in* situ and, therefore, it does not require special handling, storage or transportation of relatively dangerous concentrated chemicals. It is therefore a good alternative to the traditional sodium hypochlorite solutions.

The extent of bacterial transfer and cross-contamination from the water containing disinfectants to the washing tank surfaces (and reciprocally) that can occur during the salad washing remains poorly understood. Many organisms present in the water tend to easily adhere to the tank stainless steel surfaces and to develop communities of cells protected by a self-produced matrix of extracellular polymeric substances called biofilms (Stoodley et al., 2002). These microbial communities have the ability to live in extreme conditions and to resist cleaning procedures, being a persistent source of contamination that can lead to food spoilage (Speranza et al., 2011). Moreover, little is known regarding the biofilm behaviour after water disinfection and biofilm persistence in such food surfaces after the use of an antimicrobial. Therefore, the purpose of this study was to investigate the antimicrobial potential of NEOW when compared with the traditional sodium hypochlorite method as a water disinfecting agent at industrial scale. Additionally, this work also addresses the effect of NEOW on biofilms formed on stainless steel surfaces and their ability to regrow after disinfection.

2. Materials and methods

2.1. *Industrial scale*

2.1.1. *Sampling points and industrial scale scheme*

For NEOW production, Aqualution UK Ltd assembled the generator on site as shown in Fig. 2.

A water softener (Fig. 2, #1) and a holding tank (Fig. 2, #2) containing high-grade quality salt were mounted. The NEOW generating cell (Fig. 2, #3) had the capacity to produce 1 L of NEOW solution every 90 s.

In the NEOW generator, two types of processing water were produced (Fig. 1), electrolyzed oxidizing water (EO), which is acidic (pH 2.3–2.7), and electrolyzed reduced water (ER), which is alkaline (pH 10.0–11.5). Both solutions were mixed in the hypochlorous tank (Fig. 2, #4) in a proportion to generate a hypochlorous solution with chlorine concentration that ranged from 150 to 180 ppm whilst washing trials were performed by adjusting this concentration to 30–40 ppm of free chlorine (NEOW, pH 6.0) by means of a dosing pump (Fig. 2, #5). The NEOW solution was directly injected (Fig. 2, #9) into a closed ring of circulating water to the sanitizing tank.

The Compact Chlorometer Duo (Palintest, USA) was used for measuring free chlorine concentrations in the hypochlorous acid storage tank, and in the sanitizing tank.

Concentrated hypochlorous solution was monitored twice daily to ensure stability whilst free chlorine levels in the sanitizing tank were measured at 10-min intervals during trials.

When pH values were too low (2.5–3.0), the sodium hydroxide solution produced at the cathode side of the NEOW device was automatically added to prevent the volatilization of chlorine gas from the sanitizing tank.

At industrial scale, 400 kg of the different types of salad were washed in each trial. After a period of 20 min of chlorine concentration stabilization in sanitizing tank (4000 L water capacity), salad was fed onto the washing line.

Water samples were taken from the washing tank (only water, without antimicrobial agent) and from the sanitizing tank (Fig. 2) in three consecutive days. In the first day, NaOCl concentration ranging from 60 to 90 ppm of free chlorine was used in the sanitizing tank. The concentration of free chlorine from NEOW was 40 ppm. In the second day of sampling, NaOCl was tested as in the first day, and the chlorine concentration from NEOW was adjusted to 30 ppm. In the last day of sampling, only NaOCl in a concentration of 80 ppm was tested.

The total microbial load was determined for each sampling port. Selective media were used to quantify the diversity of microbial population in the washing and sanitizing tanks.

2.2. *Total microbial load*

Three sample volumes of 10 mL were collected aseptically in each point being serially diluted and plated in plate count agar (PCA, Merck, VWR Portugal)) according with the tracking plate technique in order to achieve 10–100 CFU per track. Plates were incubated at 30 °C for 24, 48 and 72 h.