Red Cabbage Washing with Acidic Electrolyzed Water: Effects on Microbiological and Physicochemical Properties

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Abstract: The effects of acidic electrolyzed water (AEW) on the microbiological and physicochemical properties of fresh-cut red cabbages were studied. The fresh-cut red cabbages and artificially inoculated red cabbages with *Salmonella Typhimurium* DT104 were washed with distilled water (DW) and different available chlorine concentration (ACC) of AEW for different times. AEW treatments significantly reduced the populations of native aerobic bacteria, molds and yeasts, and artificially inoculated *S. Typhimurium* DT104 compared to the DW treated and untreated red cabbage samples. The effectiveness of AEW treatments was greatly enhanced with increasing ACC and treatment times. *S. Typhimurium* DT104 were not detected in the washing water after the red cabbages treated by AEW. The surface color, pH, and total phenolic contents were did not significantly change when the red cabbages were washed with DW and 100 ppm AEW for 3 min. The anthocyanin contents and antioxidant activities of red cabbage were significantly reduced by 18.5% for cyanidin, 22.1% for pelargonidin, and 11.2% for DPPH radical scavenging activity, the impacts on the nutritional benefits of red cabbage were considered as limited and acceptable. The optimal process condition of AEW for washing red cabbage was 100 ppm for 3 min. In these conditions, most of the native microflora were inactivated, and artificial inoculated *S. Typhimurium* DT104 on the red cabbage were reduced by 40.2% (3.67 log CFU/g) and with minimal losses of nutrients and antioxidant activity, as well as no requirement of discontinuation treatment on the washing water.

Keywords: Red cabbage, acidic electrolyzed water, decontamination, *S. Typhimurium* DT104, physicochemical characteristics, anthocyanins.

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1. Introduction

Fresh-cut produce markets have increased steadily over the last twenty years worldwide, mainly due to fresh-cut produces containing higher nutrients, greater taste, and easier to prepare [1, 2]. Red cabbage is one of the world widely consumed fresh-cut vegetables with high nutritional values relating to the antioxidants and anti-inflammatory activity of anthocyanins, vitamins, and minerals [3, 4]. However, frequent incidence of food disease outbreaks associated with the consumption of fresh-cut vegetables and fruits have also been a major concerns. Therefore, using effective decontamination processes to remove bacterial contaminants in fresh-cut red cabbage is essential to ensure its safe consumption. Moreover, it is well-known that anthocyanins are very unstable and easily degrade since they are sensitive to factors like oxygen, temperature, pH, light, etc. [5-7]. Therefore, it is important to select proper treatment that in not only effective to inactivate pathogenic bacteria but also minimize the loss of nutrients and antioxidant activity of red cabbage.

Electrolyzed water is a relatively novel disinfecting solution that has been shown to effectively reduce pathogenic bacteria on fresh produces[8-11]. The acidic electrolyzed water (AEW) contains hypochlorous acid, a weak acid. It has strong oxidation potential, and a shortage of electrons giving it the ability to oxidize and destroy microbes as well as toxins. Therefore, AEW can be used as a disinfectant and antimicrobial agent[9, 12, 13]. It is well known that the treatments with AEW have many advantages in the decontaminations of vegetables and fruits including without impacts on the tissue pH, surface color, general appearance, or customer acceptance, and no off-odor or off-flavor production [14-17]. In recent years, the AEW has gained interest as a bactericidal used against a variety of microorganisms, to remove pesticide residues, and reduce mycotoxins attributes on the applications of agriculture harvest, supermarket, and food industry [10, 18, 19].

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Salmonella is the most frequently reported cause of foodborne illness and morbidity in humans. Most developed countries have various surveillance and intervention systems aimed at controlling the problem [20, 21]. It is one of serious pathogens that have been responsible for foods outbreaks in meats, ready-to-eat products, minimally processed foods, fresh produces, and fresh cut products [22, 23]. The fresh produces (vegetables and fruits) can be contaminated with *Salmonella* pathogen from environmental sources, distribution/storage, and food preparation handling process, thus the *Salmonella* must be killed on food before consumption. The emerging non-thermal technologies such as irradiation, ultraviolet light, and electrolyzed water treatments have been reported to effectively inactivate *salmonella* on fresh produces, and in some case are already applied in food industry[8, 23, 24]. Among those technologies, the AEW treatment has advantages associated with low cost, ease of operation, and remove dirt and inactivate various microbial at the same time by using AEW wash meats, vegetables, and fruits. However, the available chlorine concentration (ACC) of AEW and treatment time may greatly influence its antimicrobial activity and bioactivity of phenolic compounds present in the fresh-cut produces.

Several disinfection strategies have been studied to reduce or eliminate the microbial load of red cabbages and to ensure microbial safety. To our knowledge, there are little studies on the effects of treatment with AEW on the red cabbage. Therefore, the aim of present study was to evaluate the effects of available chlorine concentration of AEW and treatment time on the inactivation efficacy of native microflora and artificial inoculated *S. Typhimurium* DT104 on the red cabbage. The possibility of cross-contamination of processing water has also been studied. In addition, the influences of AEW on the physicochemical properties, the changes of color and pH, the total phenolic contents, anthocyanins, and antioxidant were also investigated.

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2. Results and discussion

2.1. Decontamination of aerobic bacteria and molds and yeasts on fresh-cut red cabbages surface

The effects of different available chlorine concentrations of AEW and treatment times on the efficiency of DW and AEW against aerobic bacteria, molds and yeasts on fresh-cut red cabbages are shown in Figure 1. The mean initial population of aerobic bacteria and mold and yeasts on the surface of red cabbage were 6.72±0.06 and 3.48±0.03 log CFU/g, respectively. All AEW (50, 100, and 150 ppm) treatments significantly ($p<0.5$) reduced the populations of aerobic bacteria, molds and yeasts to the range of 2.33-6.72 log CFU/g and 0.94-3.48 log CFU/g compared to the DW treatment. As the available chlorine concentrations increased from 50 ppm to 150 ppm, the reductions of aerobic bacteria and molds and yeasts were all increased. The aerobic bacteria population reductions increased from 2.33-3.85 log CFU/g to 4.72- 6.72 log CFU/g, and the molds and yeasts reductions increased from 0.94-1.98 log CFU/g to 3.48 log CFU/g. Similar results were reported when neutral electrolyzed water (NEW) were used as sanitizer for disinfecting natural flora on the shredded cabbage and carrot[25]. The disinfection efficacy of NEW was also increased with an increase of the available chlorine concentration.

As shown in Figure 1., an increase in the treatment time from 1 min to 5 min, leaded to significant reductions of the populations of aerobic bacteria, molds and yeasts for both DW and AEW.. Moreover, the AEW treatments showed a more pronounced effects of bacterial reductions compared to DW treatments. The aerobic bacteria, molds and yeasts of the red cabbages were inactivated more quickly by 150 ppm AEW than 50 and 100 ppm AEWs. When 100 ppm and 150 ppm AEW were applied for 3 min, the aerobic bacteria and molds and yeasts were not detected on

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the treated red cabbages. The molds and yeasts were also not detected on the red cabbages treated with 150 ppm AEW for 1 min.

The experimental results showed that reductions of aerobic bacteria, molds and yeasts increased with prolonging the AEW treatment times and increasing of ACC. It may be explained by the fact that hypochlorous acid (HOCl) is present in high concentration in the AEW, and it has stronger bactericidal activity [13, 26]. The germicidal action of HOCl was attributed to its penetration into microbial cells across the cell walls and the plasma membrane, leading to the inactivation of bacteria by damage to the membrane and DNA, and perhaps deterioration in membrane transport capacity that is essential for microbial growth. Increasing treatment times and ACC of AEW could provide sufficient exposure time for bacteria, molds, and yeast to react with HOCl leading to enhanced bactericidal activity of AEW. The results agreed with most of the previously reports regarding the use of AEW as disinfectant solutions for reducing the pathogen populations on the surface of vegetables and fruits by 1 to 4 log units[26-29]. When low-concentration of electrolyzed water(4 mg/L) was used to inactivate naturally occurring microflora on the organic broccoli [30], no significant difference ($P > 0.05$) were observed between treatments with electrolyzed water for 5 min and 10 min, and the reductions of the aerobic bacteria, yeasts, and molds in both groups were greater than those detected on dipping for 3 min ($P < 0.05$). Therefore, the results suggested that the washing treatment with 100 ppm AAC of AEW for 3 min was considered suitable to achieve the desired reduction in microbe numbers in red cabbages. Washing with DW alone cannot achieve the general requirement of food safety for consuming fresh cut produces.

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135 *2.2. Effects of AEW treatments with various conditions on S. Typhimurium DT104 artificially*
136 *inoculated on fresh-cut red cabbages*

137 The effects of different available chlorine concentrations (50, 100, and 150 ppm) and
138 treatment times on the efficiency of AEW against *S. Typhimurium* DT104 artificially inoculated
139 on red cabbages are shown in Figure 2. The initial population of *S. Typhimurium* DT104 artificially
140 inoculated on the red cabbages was 9.10 ± 0.01 log CFU/g. The available chlorine concentrations
141 of the AEW significantly affected the inactivation of *S. Typhimurium* on the red cabbages. The
142 results showed that the disinfection efficiency of different available chlorine concentrations of
143 AEW were significantly greater ($P < 0.05$) than DW treatments. Washing the inoculated red
144 cabbages with DW reduced *S. Typhimurium* DT104 populations by 2.02-2.46 log CFU/g, and
145 reduced by 2.44-4.06 log CFU/g when treated with different available chlorine concentrations of
146 AEW. It can be attributed to hypochlorous acid that is present in AEW produced by electrolysis,
147 and to the fact that hypochlorous acid is a source of chlorine in the AEW. The hypochlorous acid
148 inactivates bacterial cells by oxidation of cell surface sulfhydryl compounds, inactivation of
149 enzymes, and inhibition of ATP generation, consequently causing cell death. The red cabbages
150 were immersed in AEW, and AEW contacted *S. Typhimurium* DT104 on the surface of red
151 cabbages lead to hypochlorous acids diffusion into cell and inactivation of the cells. In general,
152 the diffusivity of hypochlorous acids increased with increasing concentration, and thus enhancing
153 the efficacy of inactivation. Therefore, reductions of *S. Typhimurium* DT104 increased with
154 increasing available chlorine concentrations of AEW. Similar results were published when slightly
155 acidic electrolyzed water (SAEW) was used as disinfectant for reducing inoculated levels of *S.*
156 *Typhimurium* on the oyster mushroom [31]. The SAEW treatments reduced approximately 2.08
157 log CFU/g of *S. Typhimurium* on the oyster mushroom. These results were in accordance with

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158 some studies that have reported that using AEW as disinfectant solutions reduced the *S.*
159 *Typhimurium* populations on the surface of vegetables and fruits by 2.08 to 5.00 log units [11, 31,
160 32]. As expected, the available chlorine concentrations increase from 50 ppm to 150 ppm led to
161 an increase in the *S. Typhimurium* DT104 reductions from 2.46-3.39 log CFU/g to 2.89- 3.71 log
162 CFU/g.

163 As shown in Figure 2., both AEW and DW treatments reduced the populations of *S.*
164 *Typhimurium* DT104 on the red cabbages with an increase of the treatment time from 1 min to 5
165 min. There was no statistical significant difference ($p>0.05$) between the washing in 100 ppm and
166 150 ppm AEW for 3 min and 5 min. However, the reductions in all groups were greater than those
167 detected on washing for 1 min. Along all treated red cabbages, the highest reductions of *S.*
168 *Typhimurium* DT104 were observed when the red cabbages were treated with 150 ppm AEW for
169 5 min. The exposure time plays a key role in reducing the microbial count in fresh vegetables.
170 However, increasing exposure time may cause dissociation of HOCl, and results in lower
171 antimicrobial activity of AEW. Abadias, Usall (14) reported that increasing the exposure time from
172 1 to 5 min for treatments with electrolyzed water or sodium hypochlorite did not significantly
173 affect the antimicrobial activity on different fresh-cut vegetables. The results suggested that the
174 activity of AEW was lost over a relatively short length of time.

175

176 *2.3. Microbiological properties changes of process wash water after application*

177 In fresh-cut vegetable processing industry, the maintenance of the processing wash water
178 quality is a major concern because it can be a potential source of microbial contaminations for
179 product if it had been cross-contaminated [33, 34]. To evaluate the risk of potential cross-
180 contamination, aerobic bacteria, molds, yeasts, and *S. Typhimurium* DT104 populations in

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processing water after washing fresh-cut red cabbages are shown in Table 2. Aerobic bacteria, molds, yeasts, and *S. Typhimurium* DT104 were all detected in DW after the treatments with inoculated red cabbages. Aerobic bacteria, molds and yeasts were also found in 50 ppm AEW, but not detected in 100 ppm and 150 ppm AEW. The AEW at 50, 100 and 150 ppm all reduced *S. Typhimurium* DT104 population to undetectable levels. The results indicated that *S. Typhimurium* DT104 were more sensitive to the AEW than aerobic bacteria, molds, and yeasts. This might be due to the presence of some spores in aerobic bacteria, molds and yeast [35]. The results were in agreement with the results of some studies [36, 37] that showed similar effectiveness of chlorine against pathogen. The results suggested that only the ACC of AEW up to 100 ppm, it can maintain the washing water not been cross contaminated, and may consider to be recycle and would not apply impact on the environment after discharge from industry waste, or may for further reuse.

2.4. Color changes in red cabbages after AEW treatment

Figure 3. shows the color differences (DE) between the DW-treated and AEW-treated red cabbages at different treatment times compared to the untreated control samples. The ranges of color changes (DE) in the 150 ppm AEW treated red cabbage samples were from 2.06 to 3.43, and the color changes were more noticeable than for other samples. It might due to anthocyanins degradation [3, 38]. According to Obón et al., DE under 5.0 indicates changes in color undetectable by human eye [39]. In this study, the results of all samples resulted in DE values lower than 5.0. Thus the results indicated that those treatments have not significantly changed the surface color of red cabbage. The color differences of the 50 and 100 ppm AEW treated red cabbages samples were similar (DE = 0.56-2.08), and all lower than for DW treated samples for

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204 1 min, and greater than DW treated samples for 3 to 5 min. There was no significant influence on
205 the surface color of red cabbage when the ACC of AEW was not greater than 150 ppm and the
206 treatment longer than 5 min. The results were in agreement with most of previous reports that the
207 treatments with AEW have no effects on the surface color, general appearance, or customer
208 acceptance of vegetable and fruits [14, 16]. In contrast, Lee et al. found that the colors of treated
209 cabbage changed noticeably after applying 100 ppm NEW for 30 min, and carrot for 20 min
210 [25]. They also found that the color change was noticeable in the cabbage and carrot after
211 applying 150 ppm NEW for 10 min. Therefore, the AAC and treatment time were all important
212 factors for non-sensorial changes of the vegetables. In the present study, the results suggested
213 that ACC was not in excess of 150 ppm in the AEW and the treatment times were not over 5 min
214 which enabled to maintain the sensorial quality of red cabbage. Therefore, this treatment
215 condition could be considered as appropriate for decontamination of fresh-cut red cabbage
216
217 *2.5. pH changes in red cabbages after AEW treatment*
218 The changes of pH values of the red cabbage treated with various available chlorine
219 concentrations of AEW and DW for different times are shown in Figure 4. The pH values were
220 ranging from 6.09 to 6.25. Compared to untreated red cabbage, no statistical significant ($p > 0.05$)
221 difference was observed in the pH level in DW-treated and AEW-treated(50-150 ppm) for 1-5 min.
222 Also no statistical significant difference was found in all treated samples including DW treated
223 and AEW treated (50-150 ppm) samples at different treatment times. These results are in
224 agreement with those of Eda et al. and Bessi et al.[40, 41], who reported that using EW did not
225 affect the pH of sweet cherry and dates.
226

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227 *2.6. Physicochemical properties changes in red cabbages after AEW treatment*

228 Phenolic compounds are the major antioxidants in red cabbage, and they can neutralize active
229 oxygen species and quench free radicals. Anthocyanins are red, orange, blue or purple water
230 soluble pigments occurring in fruit and vegetables[42, 43]. The high content of anthocyanins
231 contribute to purple color of red cabbage. The total phenolic compounds and anthocyanins in red
232 cabbages before and after treatments were determined, as well as the antioxidant capacity was
233 analyzed using DPPH radical scavenging activity method. The results are shown in Table 3.

234 Compared to untreated red cabbage, the treatments with high AAC (150 ppm AEW for 3 and 5
235 min) resulted in a significant decrease ($p < 0.05$) in total phenolic content. The other treatments
236 did not significantly alter ($p > 0.05$) the total phenolic content in the treated red cabbages. The
237 average total phenolic content ranged from 317.9 to 327.1 mg GAE/g. It might be attributed to the
238 oxidation property of acidic electrolyzed water, especially at high available chlorine concentration.
239 It would have caused polyphenols oxidation and degradation [44]. In contrast, Puligundla et al.
240 demonstrated that the total phenolic content of broccoli sprouts were not significantly ($P > 0.05$)
241 affected by washing with the 230 mg/ L AEW for 0-60 s compared to unwashed controls [45]. It
242 was probably due to the treatment time that was shorter than for the present study. These results
243 suggest that decontamination of fresh-cut red cabbage with 150 ppm AEW might result in a
244 negative effect on the total phenolic content.

245 Identification and quantitative analysis of anthocyanins in red cabbage before and after
246 treatments by HPLC method was performed (Table 3). The results showed that the derivatives of
247 cyanidin pelargonidin were found to be the predominant compounds, and the contents of cyanidin
248 were about 10 times more than for pelargonidin contents in red cabbages. Compared to the
249 untreated red cabbage, no statistical significant ($p > 0.05$) difference was observed on the cyanidin

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contents when the cabbages were treated with DW and 50 ppm AEW up to 5 min. However, significant decreases were observed in the red cabbages treated with 100 and 150 ppm AEW at treatment times of 3 and 5 min. The results showed that the treatments with DW, 50 , 100, and 150 ppm of AEW (3 and 5 min) all resulted in significant decrease in the contents of pelargonidin. Increasing ACC concentrations and treatment times caused decrease of anthocyanin contents. It might be attributed to the fact that anthocyanins are very unstable to processing and storage, since they are sensitive to factors like oxygen, temperature, pH, light, etc. [5, 7, 46]. Cyanidin and pelargonidin are water-soluble compound. During the treatment, the red cabbages are immersed in AEW, the oxidative AEW penetrates cell matrices resulting in the dissolution of cyanidin and pelargonidin and their oxidation in AEW. Castaneda-ovando et al. reported that anthocyanins, those with o-dihydroxyl substitution such as cyanidin, delphinidin and petunidin are the most susceptible to oxidation [47]. The DPPH radical scavenging activity results showed evidences confirming that AEW treatments significantly affected the phenolic compounds and antioxidant activity of red cabbages. No statistical significant difference was found between the untreated cabbages (17.0 ± 0.04 µmol TE/g fresh mass) and the treated cabbages with DW (17.2 ± 0.07 µmol TE/g fresh mass at 3min and 17.4 ± 0.35 µmol TE/g fresh mass at 5min) on the DPPH radical scavenging activity (µmol TE/g fresh mass). The DPPH radical scavenging activity were ranged from 17.2 ± 0.0 7 to 20.5 ± 0.19 µmol TE/g fresh mass for all treatments included DW and AEW. The control untreated samples showed 17.0±0.04 µmol TE/g fresh mass. The highest value of DPPH radical scavenging activity indicated the lowest antioxidant activity that was found on the AEW treated red cabbage samples at AAC of 150 ppm and treatment time of 5 min. The results suggested that high AAC of AEW associated with long treatment time may have negative impacts on the nutrients of red cabbage.

3. **Materials And Methods**

3.1. Materials

Fresh red cabbage and coarse salt (greater than 99.5% NaCl without additives) were purchased from local supermarket. Tryptic Soy Agar (TSA; BD Difco), Tryptic Soy Broth (TSB; BD Difco), Phosphate Buffered Saline (PBS), D/E Neutralizing broth, and Xylose lysine deoxycholate (XLD) agar (BD Difco) were obtained from Fisher Scientific Canada. DPD total Chlorine reagent and free Chlorine reagent were purchased from HACH company (Colorado, USA)Anthocyanins standards (Cyanidin Chloride and Pelargonidin Chloride) for HPLC analysis were purchased from Indofine Chemical Company Inc (Somerville, NJ). Chemical agents like 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)- S-triazine (TPTZ), Folin-Ciocalteu's phenol reagent, L-Ascorbic acid, gallic acid, formic acid, $FeCl_3$-$6H_2O$, and Na_2CO_3 were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA) and were analytic grade. HPLC-grade methanol and ethanol were purchased from Caledon Laboratories (Georgetown, Ont., Canada). Water was purified with a Mili-Q system (Millipore, Bedford, MA, USA).

3.2. Preparation of acidic electrolyzed waters and properties determination.

Brine solutions containing 5% (w/v) salt was prepared by dissolving 50 g of coarse salt into Milli-Q water for a final volume of 1L. The brine solution and distilled water were simultaneously pumped into the generator (Mini UL 50, Aquacharge Inc., Canada) to produce the electrolyzed water (EW). The flow rate of brine solution was independently adjusted to 15 mL/min by using control program on the EW generator. The Mini UL electrolyzed water generator has two modes,

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one acid-water mode and one alkaline-water mode.. The acid-water mode operation was chosen for the present study. According to Mini UL electrolyzed water generator manufacturer's instruction, the salt solution was pumped directly into the anode side during the acid-water mode of operation. The acidic electrolyzed water (AEW) was collected 5 min after starting the EW generator when the steady states of amperages were reached. The AEW was stored in a polypropylene containers with screw cap, and kept in a dark place until further use (no more than 30 hours) [48]. The properties of AEW were determined after the AEW was collected. The pH and oxidation reduction potential (ORP) of all produced AEW was measured with a dual-scale pH/ORP meter (VWR, Traceable, Canada). The total chlorine concentrations and available free chlorine concentrations were measured using a chlorine meter (CL500, Extech Instruments, Taiwan) with DPD free chlorine reagent and DPD total chlorine reagents (HACH company, Colorado, USA). Different concentrations of AEW based on the available free chlorine concentration of AEW (50, 100, and 150 ppm) were prepared by diluting AEW with sterilized distilled water. The pH, ORP and ACC of all the treatment solutions are shown in Table 1.

3.3. Preparation of inoculum

The bacterial strain used in the present work is *Salmonella enterica serovar Typhimurium* strain DT104 (strain SA970934) from porcine with antibiotic resistance to ampicillin, chloramphenicol, florfenicol, spectionomycin, streptomycin, sulfisoxazole and tetracycline [49]. From -80°C stock, *S. Typhimurium* DT104 was streaked on TSA plate, and incubated at 37°C for 24 h. The plates were stored at 4 °C for future use. A single colony was added to 75 mL of TSB in a 125 mL screwcap flask, and incubated 24 h at 37°C with shaking at 180 rpm (MaxQ 420 HP, Thermo Science, ON, Canada). After 24 h of incubation, 35 mL of cultures was added into each one of two

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50 mL centrifuge tube and pelleted by centrifugation at 7000 rpm for 10 min (Sorvall RT1, Thermo Science, ON, Canada). The resulting pellets were washed in 35 mL of PBS and centrifuged at 7000 rpm for 10 min. The resulting pellets were resuspended in 7 mL of PBS each and vortexed. Then the two suspensions were combined together and vortexed for homogenization. These suspensions were used as inocula of fresh red cabbages. The population of inocula was approximately 9.0 log CFU/g.

3.4. Preparation and inoculation of red cabbage

The damaged outer leaves of red cabbage were removed and disposed, while the rest of leaves were cut into approximately 0.5×3 cm segments using a sharp knife, and placed in a sterile polyethylene bag and mixed. Twenty grams leaves was randomly weighed for each sample, and put on a sterile polyethylene square (15 cm x 15cm) in the biological safety cabinet (BSC). Spot inoculation was performed with a micropipette by transferring 1 mL (approximately 20 drops) of the well-mixed bacteria suspension randomly onto leaf surfaces of all samples except the negative control. The bacterial suspensions on the leaf surfaces were dried for 2 hours inside BSC.

3.5. Treatments and microbiological analysis

After spots inoculation and drying, the inoculated red cabbage leaves were transferred into sterile stomacher filter bags (FILTER-BAG ®, VWR, Pennsylvania, USA). One hundred eighty millilitres of AEW containing 50 ppm (EW50), 100 ppm (EW100), or 150 ppm (EW150) ACC was added into the bags individually and treated for 1, 3, or 5 min. The inoculated and non-inoculated red cabbages treated with sterile distilled water (DW) served as positive and negative controls respectively. During the treatments, the bags were sealed by clips and manually shaken

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342 vigorously in order to ensure all leaves were immersed in AEW or DW. After treatments for 1, 3,
343 or 5 min, the AEW was poured in a sterilized bottle for microbial analysis. Then 180 mL of sterile
344 saline solutions (0.85% NaCl) were added into the stomacher bags with treated red cabbages.
345 Subsequently, the bagged red cabbage samples were homogenized for 1 min in a paddle blender
346 at 260 rpm (Seward Stomacher 400, London, England). The homogenates were serially diluted in
347 sterile D/E broth, and 100 µL aliquots of diluent were spread-plated on TSA agar in duplicate. The
348 plates were dried with the lid open in the BSC until no visible diluent seen. Subsequently the plates
349 were incubated at 37 °C for 2 h, XLD was poured on the top of TSA agar to create a bilayer plate,
350 and dried them again in BSC. The plates were then incubated at 37 °C for 24 h. The colonies were
351 counted and expressed as $\log_{10}$ colony forming units per gram (log CFU/g). The initial population
352 of *S. Typhimurium* DT104 on the surface of red cabbage were determined by a series of 10-fold
353 dilutions made in D/E broth directly, and 100 µL was spread-plated on XLD agar, and incubated
354 at 37°C for 24 h.

355 The total natural microbial loads including aerobic bacteria, molds, and yeasts were also
356 determined on the treated cabbage samples that followed the same treatments of AEW and DW
357 (control) as described previously for *S. Typhimurium*. The selective agars were used to quantify
358 the diversity of microbial population in the cut red cabbage leaves before and after treatment. The
359 TSA for aerobic bacteria and incubated at 37 °C for 2 h, and the potato dextrose agar (PDA) for
360 growing molds and yeasts at room temperature for one week in the dark place.

361 The washing water samples were analyzed using the same enumeration methods than for *S.*
362 *Typhimurium* and were expressed as log10 colony forming units per mL (log CFU/g). Three
363 replicates of each experiment were carried out.

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365 *3.6. Effects of AEW treatments on the quality of red cabbages*

366 **3.6.1 The changes of color**

367 The surface color changes of the red cabbage were measured according to the Lee et al.
368 method [25]. A HunterLab LabScan XE spectrocolorimeter (Hunter Associates Laboratory, Inc.,
369 Reston, VA, USA) was used to determine the color changes of red cabbage samples in the
370 CIELAB scale with the EasyMatchQC software. Samples were measured in triplicates and
371 results was expressed as values of L, a and b, for darkness/brightness, redness/greenness and
372 yellowness/ blueness. The net color difference (DE) was calculated as follows:

373 Color difference (DE) = $\sqrt{(\boldsymbol{L}-\boldsymbol{L_0})^2+(\boldsymbol{a}-\boldsymbol{a_0})^2+(\boldsymbol{b}-\boldsymbol{b_0})^2}$

374 L/L_0 L values for the treated/untreated samples.

375 a/a_0 a values for the treated/untreated samples.

376 b/b_0 b values for the treated/untreated samples

377

378 3.6.2 The changes of pH

379 pH of red cabbages were determined according to the previously given method[25]. Ten grams
380 of the red cabbage samples were treated with 90 mL AEW (50 ppm, 100 ppm, 150 ppm) for 1, 3,
381 and 5 min in a beaker to ensure that the leaves were fully immersed. After treatment, the treated
382 leaves were drained from AEW and surface water was removed using paper towel. Leaves were
383 cut into small pieces and ground with 50 mL of deionized water using a grinder (Magic Bullet, CA
384 USA) for 1 min. The pH of the homogenates were measured at 23°C using a pH meter with glass
385 electrode (Orion Star A111, Thermo Scientific, Indonesia). The leaves treated with DW were used
386 as control and pH of DW was considered as control reference to compare the differences between
387 DW and AEW treated samples.

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388

389 *3.7. Analysis of physicochemical properties*

390 3.7.1 Extraction procedures

391 After treatments with AEW (50 ppm, 100 ppm, and 150 ppm) and DW for 3 and 5 min, the
392 treated leaves (10g) were drained from AEW or DW, and surface water were removed using paper
393 towel. The treated leaves were cut into small pieces, and ground with 25 mL 80% ethanol and 0.35
394 µL formic acid using a grinder (Magic Bullet, CA USA) for 1 min. The extracts were obtained by
395 filtering the samples under vacuum to remove the mashed leaves. The filtrates were then passed
396 through 0.45 m filters, and were immediately subject to anthocyanins (HPLC method), total
397 phenolic, and DPPH radical scavenging activity analysis. The untreated red cabbage leaves were
398 used as control samples, and extracted and analyzed following the same procedures.

399 3.7.2 Total phenolic contents.

400 The effects of AEW on the total phenolic contents of red cabbages were determined by
401 colorimetric method with 96-well microplate based on Folin-Ciocalteu's phenol reagent (FCR)
402 [50]. The values are expressed as mg gallic acid equivalents (GAE) per gram of extract. Twenty
403 five microliters aliquots of various GAE standards (0.03125-0.5 mg/mL) and the diluted red
404 cabbage extracts (8 times) were transferred to the appropriate wells in triplicates, and H_2O as a
405 blank. 125 µL of 1/10 diluted FCR were added into each of the wells, the samples were left for 8
406 min. 125 µL7.5% Na_2CO_3 were pipetted into each of the wells, and were kept at room temperature
407 for 1 h in dark place. Then, the absorbance was recorded against a blank at 725 nm using a
408 microplate reader (BioTek Instruments, VT. USA)

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410 3.7.3 Determination of anthocyanins

411 Identification and quantitative analysis of anthocyanins was carried out using HPLC (Agilent
412 1100 series system, Agilent Technologies) equipped with a photodiode-array detector (Agilent
413 Technologies, Waldbornn, Germany). The analytical column employed was a C-18 (150 mm × 4.6
414 mm i.d. × 5 uL) column manufactured by Dikma, Inc. The temperature of the column oven was
415 25°C. Gradient elution was performed using 10% formic acid (solution A) and 100% methanol
416 (solution B) as follows: linear gradient from 95% A/5% B to 40% A/60% B, 0-20 min; isocratic
417 elution 40% A/60% B, 20-23 min; linear gradient from 40% A/60% B to 95% A/5% B, 23-24 min;
418 Isocratic elution 95% A/5% B, 24-28 min. The flow rate was 0.7 mL/min, and the injection volume
419 was 20 uL for all samples and standard solutions. The wavelengths used for quantification were
420 520 nm. The standard curves were plotted for each compounds based on the peak area vs known
421 concentrations from 0.5-0.0625 mg/mL.

422 3.7.4 Determination of 2,2 diphenyl-1-1picrylhydrazyl (DPPH) radical scavenging activity

423 Promising antioxidant activity of the treated red cabbages before and after AEWs and DWs were
424 evaluated by using a model of scavenging the stable DPPH radicals scavenging activity [51]. A
425 96-well microplate was used for DPPH assay. Ten microliters of red cabbage extracts and standard
426 solutions (62.5-1000 uM AA) were transferred to appropriate wells in triplicates, then 200 µL
427 DPPH (350 µM) were added into each well. Plates were kept in a dark place at ambient temperature
428 for 6 h before the absorbance (A) at 517 nm was measured by using a microplate reader (BioTek
429 Instruments, VT. USA). A standard curve was plot using 62.5-1000 µmol L^{-1} Trolox solutions
430 with $R^2 = 0.99$. Methanol without DPPH was used as the blank. Methanol with DPPH was used as
431 control sample. Antioxidant activity was expressed as µmol/ L Trolox equivalent (TE) /g red
432 cabbage.

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433

434 *3.8. Statistical analysis*

435 All experiments and analysis were implemented in triplicate, and the data represented as the
436 mean values with standard deviation. The significance difference was measured at confidence level
437 of 95% ($p < 0.05$). Data were subjected to analysis of variance and Duncan's multiple range tests
438 using SPSS v.20.0 software (SPSS Inc., USA).

439

440 **4. Conclusion**

441

442 In the present study, the effects of available chlorine concentration of AEW and treatment time
443 on the inactivation efficacy of native microflora and artificial inoculated *S. Typhimurium* DT104
444 on the red cabbage and in the process washing water were evaluated. The distilled water (DW)
445 treatments did not show statistical significant effects on the inactivation of all microbial cells. In
446 contrast, after the red cabbages treated by AEW, the populations of native aerobic bacteria, and
447 molds and yeasts, on the surfaces of red cabbage were significantly reduced 2.33-6.72 log CFU/g,
448 0.94-3.48 log CFU/g, and respectively. The most suitable available chlorine concentration of
449 AEW for washing red cabbage was 100 ppm for 3 min. In this optimal process conditions, most
450 of the native microflora were inactivated, and artificial inoculated *S. Typhimurium* DT104 on the
451 red cabbage were reduced by 40.2% (3.67 log CFU/g) , also the washing water has not been cross
452 contaminated, and could be recycled. The surface color, pH and total phenolic contents of red
453 cabbage were not significantly affected by 3 min washing with the 100 ppm of AEW. Although
454 the anthocyanins contents and antioxidant activities of red cabbage were significantly reduced by
455 18.5% for cyanidin, and 22.1% for pelargonidin, and 11.2% for DPPH radical scavenging activity,

456 but the impacts on the nutritional benefits of red cabbage were considered as limited and acceptable.
457 Therefore, 100 ppm of AEW washing solution can be used as an alternative environmentally-
458 friendly sanitizer in fresh-cut food industry to prolong the red cabbage shelf life. The results
459 generated from the present study could provide useful information to the fresh produces and the
460 fresh-cut produce industry to optimize processing conditions, or may be combined with other
461 technologies, such as ultrasound, mild heat, and active packaging to enhance the effectiveness of
462 AEW treatment on the inactivation of pathogens and prevention of nutrients losses.
463

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Highlights

- The first paper, to study the effect of treatment with AEW on the microbiological and physicochemical properties of fresh-cut red cabbages
- The first paper, to study the effect of available chlorine concentration of AEW and treatment time on AEW final properties.
- Red cabbage physicochemical and sensory qualities unaffected by 100 ppm AEW washing for 3 min.
- AEW has the potential for improvement of red cabbage microbial quality.

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627 **Figure captures**

628 • Figure 1. Reductions (log CFU/g) of aerobic bacteria, molds and yeasts on fresh-cut red
629 cabbages after AEW and distilled water treatments for different times

630 • Figure 2. *S. Typhimurium* reduction (log CFU/g) in fresh-cut red cabbages after AEW
631 treatment

632 • Figure 3. Effect of DW and acidic electrolyzed water (AEW) at various available chlorine
633 concentration on color differences (DE) of red cabbage

634 • Figure 4. pH of fresh-cut red cabbages after DW and AEW treatment

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637 **Table 1. Physicochemical properties of the acidic electrolyzed water used in the experiments.**

638

Treatment	Total chlorine conc. (ppm)	pH	ORP (mv)
DW	0.01	7.01	265.3
AEW50 ppm	50.7	3.94	938.3
AEW100 ppm	100.5	3.57	995.7
AEW150 ppm	150.6	3.31	1020

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639 **Table 2 .Aerobic bacteria, molds, yeasts, and S. Typhimurium DT104 (log CFU/g) in**
640 **processing water after washing fresh-cut red cabbages.**

ACC	Aerobic bacteria			Mold and yeast			*S. Typhimurium* DT104		
(ppm)	1 min	3 min	5 min	1 min	3 min	5 min	1 min	3 min	5 min
DW	5.45±0.03	5.32±0.05	5.46±0.07	3.38±0.07	3.39±0.02	3.22±0.03	7.64±0.18	7.91±0.03	7.96±0.04
50	2.30±0.05	2.18±0.01	ND	2.54±0.01	2.0±0.01	1.7±0.01	ND	ND	ND
100	ND	ND	ND	ND	ND	ND	ND	ND	ND
150	ND	ND	ND	ND	ND	ND	ND	ND	ND

641 ND: not detected (below the limit of detection of 1.0 CFU/g).

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642 **Table 3 Physicochemical characteristics of red cabbages treated with DW and AEW.**

ACC (ppm)	Treatment time (min)	Total phenolic content (mg GAE/g)	Anthocyanin (μg /g)		DPPH-scavenging activity (μmol L^{-1} TE g^{-1})
			Cyanidin	Pelargonidin	
Control	0	327.1 ± 1.0 c	57.9 ± 1.1 d	6.8 ± 0.3 e	17.0 ± 0.04 a
DW	3	326.1 ± 1.0 c	53.2 ± 2.8 d	6.1 ± 0.2 d	17.2 ± 0.07 a
	5	325.5 ± 1.6 c	52.4 ± 0.7 cd	6.2 ± 0.1 d	17.4 ± 0.35 ab
50	3	326.7 ± 4.4 c	50.8 ± 2.4 bcd	6.0 ± 0.2 d	18.1 ± 0.03 bc
	5	322.1 ± 1.6 abc	44.9 ± 1.5 bcd	5.4 ± 0.2 cd	18.6 ± 0.42 cd
100	3	324.0 ± 1.8 bc	47.2 ± 2.0 abc	5.3 ± 0.2 c	18.9 ± 0.14 d
	5	321.0 ± 2.2 abc	42.2 ± 2.1 ab	4.5 ± 0.3 b	19.8 ± 0.21 e
150	3	319.2 ± 2.5 ab	45.2 ± 0.6 ab	5.0 ± 0.2 bc	19.9 ± 0.02 e
	5	317.9 ± 1.8 a	37.9 ± 1.4 a	4.4 ± 0.1 a	20.5 ± 0.19 e

643 Mean values in the same column with different letters are significantly different ($p < 0.05$).

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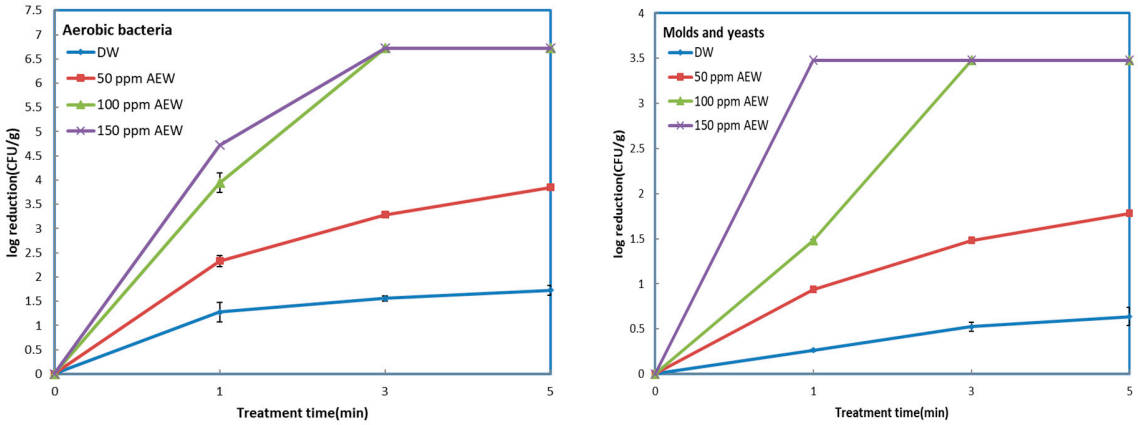

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648 Figure1. Reductions (log CFU/g) of aerobic bacteria, molds and yeasts on fresh-cut red cabbages
649 after AEW and distilled water treatments for different times

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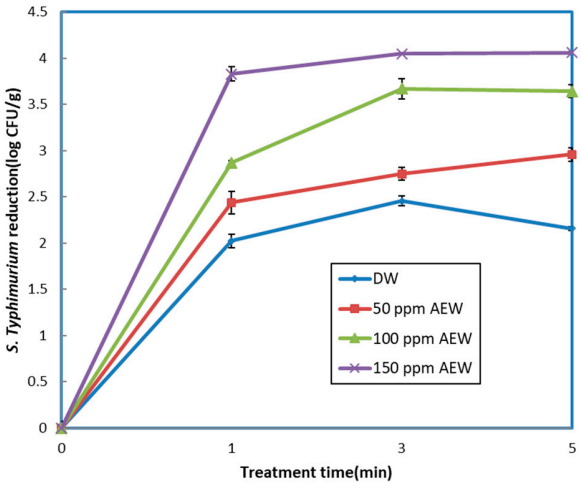

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652 Figure 2. *S. Typhimurium* reduction (log CFU/g) in fresh-cut red cabbages after AEW treatment

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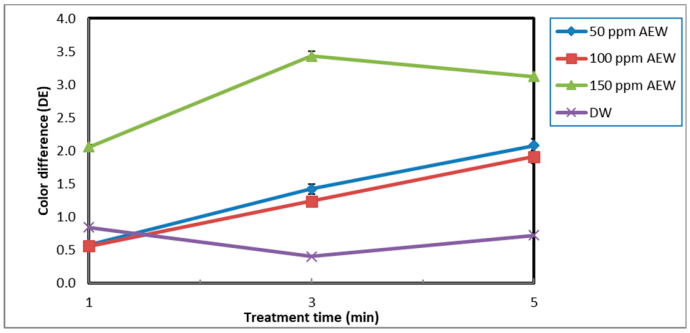

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659 Figure 3. Effect of DW and acidic electrolyzed water (AEW) at various available chlorine

660 concentration on color differences (DE) of red cabbage

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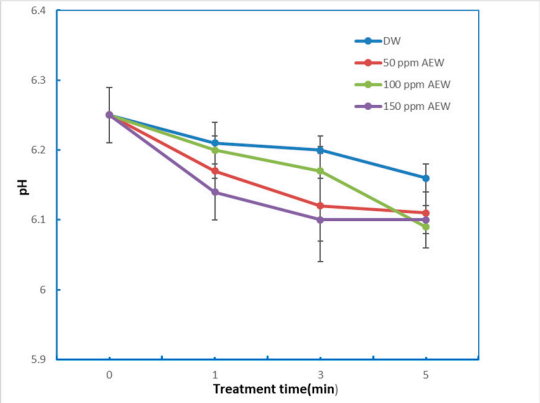

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666 Figure 4. pH of fresh-cut red cabbages after DW and AEW treatment