International Proceedings of Chemical, Biological and Environmental Engineering, Vol.95 (2016)
DOI:10.7763/IPCBEE.2016.V95.10

The Application of Slightly Acidic Electrolyzed Water as a Potential Washing Agent on Shelf-life and Quality of Fresh Cut Vegetables (Lettuce and Carrot)

Liping Wang [1], Qiang Xia [1], Peng Huang [1] and Yunfei Li [1+]

[1] Department of Food Science and Technology, School of Agriculture and Biology, Shanghai Jiao Tong University

Abstract. In order to evaluate slightly acidic electrolyzed water (SAEW) and sodium hypochlorite solution, the washing agents on shelf-life and quality were investigated during 25 days cold storage. The results showed that the specific maximum peak force of lettuce and carrot significantly increased after treated with SAEW, while carrot with sodium hypochlorite solution treatment was not significantly ($P > 0.05$) increased. Also the shelf-life of lettuce processed with SAEW was prolonged for another 4.5 days. The results indicated that SAEW technology had stronger decontamination ability than sodium hypochlorite with its conveniences.

Keywords: slightly acidic electrolyzed water, microbiology, texture.

1. Introduction

Due to the disruption of surface cells and the injury of tissues, the fresh-cut vegetables are more susceptible to microbial spoilage and pathogen contamination than raw materials [1]. Recently, minimally processed vegetables have become more and more popular, which promote the research on quality of fresh-cut vegetables, including nutrition, texture and microbiology. The objective of post-harvest washing attempts to reduce microbial loading and cross-contamination. Chemical methods including compounds of chlorine and acidic electrolyzed water are usually used for retaining nutrition and sensory, and reducing microbial loading of the processed vegetable [2].

Acidic electrolyzed water was initially developed in Japan [3] and has been considered as an effective antimicrobial agent over the last decades. According to the available chlorine content (ACC) and pH, acidic electrolyzed water was divided into strong acidic electrolyzed water (pH≤2.7，ACC 20~60 mg/kg) and slightly acidic electrolyzed water (pH 5.0~6.5，ACC 10~30 mg/kg) [4], [5]. Al-Holy and Rasco [6] demonstrated that acidic electrolyzed water could considerably reduce common foodborne pathogens in fish, chicken and beef products, like Escherichia coli O157:H7, Salmonella Typhimurium and Listeria monocytogenes. The antimicrobial effects of acidic electrolyzed water mainly rely on low pH, high oxidation reduction potential (ORP) and ACC to destroy microbial cell membrane [7].

Slightly acidic electrolyzed water (SAEW) is drawing the attention for its strong antimicrobial activity [8]. SAEW, also named neutral electrolyzed water or low concentration electrolyzed water, has gained much more attention because it has strong antimicrobial activity, non-corrosion of processing equipment, less side effect to human health and environment [1], [8]. The antimicrobial effect of SAEW at low ACC and a near neutral pH was similar to strong acidic electrolyzed water and sodium hypochlorite solution [5], [7]. Studies have shown that the bactericidal activity of SAEW is attributed to a high concentration of hypochlorous acid (HOCl; approximately 95%) [9]. Koide et al [10] indicated that mildly heated SAEW treatment reduced the total aerobic bacteria by 2.2 log CFU/g compared with tap water and had insignificant differences in

+ Corresponding author. Tel.: +86 21 34206918; fax: +86 21 34206918.
E-mail address: yfli@sjtu.edu.cn.

hardness, ascorbic acid and β-carotene contents of sliced carrots. This study aimed at investigating the effect of SAEW on reducing microbial loads, quality of fresh-cut vegetables after the experiment and compared with sodium hypochlorite solution.

2. Materials and Methods

2.1. Sample Preparation

Lettuces and carrots were purchased at Cangyuan road Farmer's market (Shanghai, China). The samples were selected only for similar size and color. For lettuce, core and outer of leaves were hand removed and discarded while the leaves were cut into pieces of 20 ± 1 mm in length with a stainless steel knife. While the carrots firstly were cut into 10 ± 1 mm wafers, then the slices were put in a mold to shape as cylinders (diameter: 12 mm). The prepared samples were well mixed and randomly divided into four groups, respectively.

2.2. Generation of Washing Agents

The sodium hypochlorite solutions were prepared by adding the appropriate volume of a concentrated solution of sodium hypochlorite (NaClO, Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) to tap water and the value of pH was adjusted to nearly 5.5 using hydrochloric acid (HCl) [11]. SAEW was generated using a flow type electrolysis apparatus (Prosperity Prosperous Sanitary Products Co., Ltd, Shanghai, China). The flow rate of water was 3.5 L/min, the electric current was 1.0 A. The washing agents of pH and ORP were determined using a pH and ORP meter (Five Easy Mettler-Toledo Instruments Co., Ltd, Schwerzenbach, Switzerland). Also the ACC was measured using a chlorine test kit (detection limit of free chlorine: 200 mg/L; HI96771; Hana Instruments, Italy).

2.3. Microbiological Analysis

To enumerate the microorganisms, 10 g of each sample combined with 90 ml normal saline (0.85 g NaCl /100 g distilled water) in a sterile stomacher blender bags was homogenized for 120 s at 8 times/SEC by a homogenizer (Model BM-400HP, Truelab Instruments Co., Ltd, Shanghai, China). Further, the aliquot was used for various serial dilutions. After decimal dilutions, one mL of suitable dilutions was pour plated on plate count agar (PCA; Huankai Company, Guangdong, China) and plates were enumerated after incubation at 37 ℃ for 48 h for obtaining total microbial count [8]. A group of three packaged samples with the same treatment were considered and analyzed to achieve the results. Plate's containing 30-300 colony-forming units (CFU) were selected for counting, and results were expressed as the log of colony forming unit per gram of samples (log CFU/g). If plates' CFUs of all dilution degrees was not in the range of 30-300, the CFUs closer to 30 or 300 was calculated. Detection limit was 1.0 log CFU/g. When no colonies were detected, an arbitrary value of 0.5 log CFU/g was marked. All samples were analyzed in triplicate.

2.4. Texture Determination

Firmness of lettuce and carrot processed with washing agents was measured with a texture analyzer (TA-XT Plus, Stable Micro Systems Ltd., Godalming, UK) for shear-compression testing [12]. Trigger force was 5 g, and the strain rate of penetration was 90%. Pretest speed was set at 2 mm/s, test speed at 1 mm/s and post-test speed at 2 mm/s. Texture measurements were performed on days 0, 5, 10, 15, 20, 25 with 8 replicates per bag. Maximum peak force was calculated for brittleness. In addition, the leaf of lettuce was folded into two layers for test.

2.5. Statistical Analysis of Data

Three replicated trials were performed for each experiment. Statistical analysis was analyzed by SAS9.1 (SAS Institute Inc., North Carolina, USA), and the methods for determination of significant difference were T TEST and ANOVA processing. The value of least significance difference was 0.05, and data were expressed as mean ± standard deviation (SD).

3. Results and Discussion

3.1. Physicochemical Properties of Washing Agents

The properties (ACC, pH and ORP) of washing agents (SAEW and sodium hypochlorite solution) used in this study are presented in Table 1, and the effects of washing agents against fresh-cut vegetables (lettuce and carrot) were observed in this study. The ACC values for SAEW and sodium hypochlorite were 30.0 ± 1.0 and 28.7 ± 1.5, the pH values for SAEW and sodium hypochlorite were 5.65 ± 0.06 and 5.56 ± 0.04. And there was no significant difference between SAEW and sodium hypochlorite solution for the value of ACC and pH. However, the value of ORP showed a significant difference between SAEW and sodium hypochlorite solution, and SAEW showed a higher ORP.

Table 1: Physicochemical properties of washing agents

Washing agents	ACC (ppm)	pH	ORP (mV)
SAEW	30.0 ± 1.0a	5.65 ± 0.06a	935.0 ± 5.0a
sodium hypochlorite solution	28.7 ± 1.5a	5.56 ± 0.04a	918.3 ± 7.6b

Note: Values were the means of three replicated mean ±standard deviation (SD); the different letters in the same row indicated significant differences ($P < 0.05$). SAEW means slightly acidic electrolyzed water, ACC means available chlorine content.

3.2. Effects of Washing Agents on Total Microbial Populations of Lettuce and Carrot during Chilled Storage

Changes of washing agents on total microbial populations for lettuce and carrot during chilled storage are shown in Fig. 1. All microbial growth curves were four periods, including lag phase, generation growth phase, stable stage and deadline phase. After treatment, the microbial load of lettuce was higher than carrot's. The initial contaminating bacteria count of lettuce was more than carrot, and the data was not shown. For lettuce, microbial load of samples processed with SAEW was lower than that of sodium hypochlorite, which can be attributed to the high ORP value of SAEW. The oxidation reduction potential (ORP) of SAEW is relatively high, and the ORP of solutions was an indicator of its ability to oxidize or reduce [6]. The optimum ORP value for growth of aerobic microorganisms is +200 to +800 mV, the favored range for growth of anaerobic microorganisms is -30 to -550 mV, and the optimal range for growth of most facultative anaerobes is +200 and -250 mV (Jay et al., 2005). The ORP of washing agents was more than 900 mV, and had significant difference ($P<0.05$), which accounted for the less microbial loads after treated with SAEW compared with sodium hypochlorite. Since the ORP played an important role in killing microorganisms. The mechanisms of SAEW on inactivating bacteria were included inactivating an enzyme of the bacterium [13] and improving the bacterial membrane permeability [14]. Thus, these effects could cause the rise of conductivities and the rapid leakages of intracellular content, including DNA, K^+, and protein. Sodium hypochlorite can be used as a disinfectant, while it is difficult to adjust the pH and ACC. The great advantage of SAEW to sodium hypochlorite is the produced SAEW with a stable pH and ACC [13].

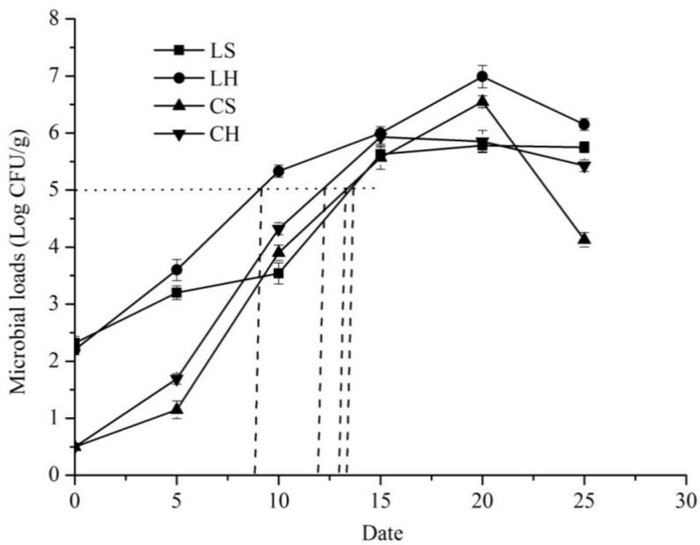

Fig. 1: Changes of washing agents on total microbial populations for fresh-cut vegetables (lettuce and carrot) treated with SAEW and sodium hypochlorite solution during chilled storage. LS means lettuce washed by SAEW, LH means lettuce washed by sodium hypochlorite, CS means carrot washed by SAEW, CH means carrot washed by sodium hypochlorite.

The microbial growth of carrot showed a similar trend. Shelf-life is defined as the total microbial loads of fresh-cut vegetables increased by 5 log CFU/g [15]. During 4℃ refrigerator storage, shelf-life of lettuce processed with SAEW and sodium hypochlorite were about 13.5 and 9.0 days, respectively; While the shelf-life of carrot were 13.0 and 12.0 days, respectively. Generally, the microbial population > 7 log CFU/g on foods is considered to represent the initial stage of spoilage [15]. Thus, after chilled storage for 20 days, samples treated with washing agents would start spoilage phase.

3.3. Effects of Washing Agents on Texture of Lettuce and Carrot during Chilled Storage

In this study, textures of fresh-cut lettuce and carrot after washed with SAEW and sodium hypochlorite solution during chilled storage are shown in Fig. 2 and Fig. 3. The texture was designed to mimic the chewing motion. For lettuce, in all samples, specific maximum peak force significantly increased after treated with SAEW and sodium hypochlorite solution, and the maximum value exhibited in day 15. For carrot, specific maximum peak force significantly increased after treated with SAEW, while it not significantly increased after treated with sodium hypochlorite. The results indicated that the increase in firmness was because of dehydration and the onset of lignifications during storage [12]. In addition, fresh-cut vegetables (lettuce and carrot) treated with SAEW and sodium hypochlorite exhibited significant difference. It was likely that samples processed by SAEW had stronger permeability than that of washed with sodium hypochlorite.

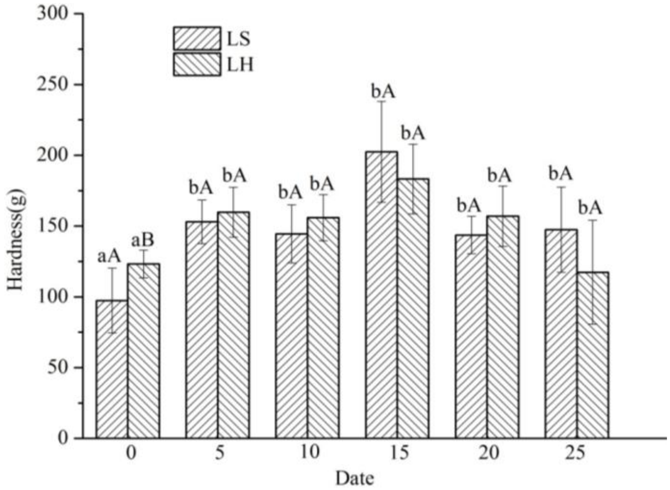

Fig. 2: The texture of fresh-cut lettuce treated with SAEW and sodium hypochlorite solution during chilled storage. LS means lettuce washed by SAEW, LH means lettuce washed by sodium hypochlorite solution; the lowcase letters a, b means the texture had significant difference with time passing ($P < 0.05$); the capital letters A, B means the texture had significant difference between washing with SAEW and sodium hypochlorite solution ($P < 0.05$).

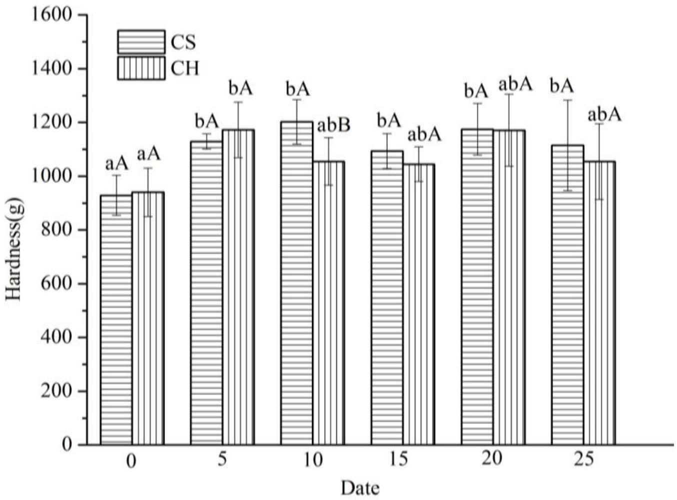

Fig. 3: The texture of fresh-cut carrot treated with SAEW and sodium hypochlorite solution during chilled storage. CS means carrot washed by SAEW, CH means carrot washed by sodium hypochlorite solution; the lowcase letters a, b means the texture had significant difference with time passing ($P < 0.05$); the capital letters A, B means the texture had significant difference between washing with SAEW and sodium hypochlorite solution ($P < 0.05$).

4. Conclusion

In conclusion, the results of this research revealed that SAEW can be used as an alternative washing agents for reducing microbial load and extending shelf-life in some degree. SAEW as a promising washing agent, provided an environmentally effective approach on the processing of fresh-cut vegetables for its antimicrobial activity, non-corrosion of processing equipment, less side effect to human health and environment [1], [8], and required further research.

5. Acknowledgements

The research was supported by the Project of Prepared Food Research and Industrialization in City Nutrition Catering (No. 2014BAD04B00).

6. References

[1] J. Hao, H. Li, Y. Wan and H. Liu. Combined effect of acidic electrolyzed water (AcEW) and alkaline electrolyzed water (AlEW) on the microbial reduction of fresh-cut cilantro. *Food Control.* 2015, **50**: 699-704.

[2] A. Tomás-Callejas, F. López-Gálvez, A. Sbodio, F. Artés, F. Artés-Hernández and T. V. Suslow. Chlorine dioxide and chlorine effectiveness to prevent Escherichia coli O157:H7 and Salmonella cross-contamination on fresh-cut Red Chard. *Food Control.* 2012, **23** (2): 325-332.

[3] Y. Shimizu and T. Hurusawa. Antiviral, antibacterial, and antifungal actions of electrolyzed oxidizing water through electrolysis. *Dental. J.* 1992, **37**: 1055-1062.

[4] J. L. Guentzel, K. Liang Lam, M. A. Callan, S. A. Emmons and V. L. Dunham. Reduction of bacteria on spinach, lettuce, and surfaces in food service areas using neutral electrolyzed oxidizing water. *Food Microbiology*. 2008, **25** (1): 36-41.

[5] A. Issa-Zacharia, Y. Kamitani, N. Miwa, H. Muhimbula and K. Iwasaki. Application of slightly acidic electrolyzed water as a potential non-thermal food sanitizer for decontamination of fresh ready-to-eat vegetables and sprouts. *Food Control.* 2011, **22**(3): 601-607.

[6] M. A. Al-Holy and B. A. Rasco. The bactericidal activity of acidic electrolyzed oxidizing water against Escherichia coli O157:H7, Salmonella Typhimurium, and Listeria monocytogenes on raw fish, chicken and beef surfaces. *Food Control.* 2015, **54**: 317-321.

[7] S. M. E. Rahman, T. Ding and D.-H. Oh. Inactivation effect of newly developed low concentration electrolyzed water and other sanitizers against microorganisms on spinach. *Food Control.* 2010, **21**(10): 1383-1387.

[8] T. Ding, Z. Ge, J. Shi, Y.-T. Xu, C. L. Jones and D.-H. Liu. Impact of slightly acidic electrolyzed water (SAEW) and ultrasound on microbial loads and quality of fresh fruits. *LWT-Food Sci. Technol.* 2015, **60** (2): 1195-1199.

[9] C. Zhang, Z. Lu, Y. Li, Y. Shang, G. Zhang and W. Cao. Reduction of Escherichia coli O157:H7 and Salmonella enteritidis on mung bean seeds and sprouts by slightly acidic electrolyzed water. *Food Control.* 2011, **22**(5): 792-796.

[10] S. Koide, D. Shitanda, M. Note, W. Cao, Effects of mildly heated, slightly acidic electrolyzed water on the disinfection and physicochemical properties of sliced carrot. *Food Control.* 2011, **22** (3): 452-456.

[11] F. López-Gálvez, A. Allende, P. Truchado, A. Martínez-Sánchez, J. A. Tudela, M. V. Selma and M. I. Gil. Suitability of aqueous chlorine dioxide versus sodium hypochlorite as an effective sanitizer for preserving quality of fresh-cut lettuce while avoiding by-product formation. *Postharvest Biol. Technol.* 2010, **55** (1), 53-60.

[12] R. G. Klaiber, S. Baur, G. Wolf, W. P. Hammes and R. Carle. Quality of minimally processed carrots as affected by warm water washing and chlorination. *Innov. Food Sci. Emerg. Technol.* 2005, **6**(3), 351-362.

[13] H. Kiura, K. Sano, S. Morimatsu, T. Nakano, C. Morita, M. Yamaguchi, T. Maeda and Y. Katsuoka. Bactericidal activity of electrolyzed acid water from solution containing sodium chloride at low concentration, in comparison with that at high concentration. *J. Microbiol. Meth.* 2002, **49** (3), 285-293.

[14] X. Zeng, W. Tang, G. Ye, T. Ouyang, L. Tian, Y. Ni and P. Li. Studies on disinfection mechanism of electrolyzed oxidizing water on E. coli and Staphylococcus aureus. *J. Food Sci.* 2010, **75** (5), M253-M260.

[15] S. Koseki, K. Itoh, Prediction of microbial growth in fresh-cut vegetables treated with acidic electrolyzed water during storage under various temperature conditions. *Journal of Food Protection®*. 2001, **64** (12): 1935-1942.