Environmentally Friendly and Safe Technologies for Quality of Fruits and Vegetables

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The papers contained in this book report some of the peer reviewed Proceedings of the International Conference "Environmentally friendly and safe technologies for quality of fruit and vegetables", but also other papers related with the subject were included. The manuscripts were reviewed by the Editor and Editorial Board, and only those papers judged suitable for publication were accepted. The Editor wish to thank to all the reviewers and authors for their contribution.

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SECTION 5. NEW APPROACHES TO ENHANCE SAFETY AND QUALITY OF MINIMALLY PROCESSED FRUITS AND VEGETABLES

34. EFFECT OF ELECTROLYZED WATER AS A DISINFECTANT FOR FRESH-CUT FRUIT

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Abstract

In fresh-cut processing the disinfection is the most important step. This process can affect the quality and the safety of the end product as well as its shelf life. Chlorine is the most used disinfectant in fresh-cut industry but due to its association with environmental and health risks, the development of alternative sanitizers is emergent. Electrolyzed water (EW) may be an alternative to the use of chlorine sanitizers and both acidic electrolyzed water (AEW) and neutral electrolyzed water (NEW) can be used with this purpose. In fact, several studies have shown that EW can be effective in reducing pathogenic bacteria on the surface of fruits and vegetables. This manuscript presents a review of electrolyzed water approaches, its antimicrobial activity in fresh-cut produce and the results of a study undertaken in our laboratory to evaluate the disinfection efficacy of AEW and NEW on fresh-cut apples and oranges. Fresh-cut apples and fresh cut oranges were inoculated with a suspension of *Listeria innocua* and fresh-cut oranges with *Escherichia coli* at a concentration of 10^7 cfu mL^{-1} and treated with the different solutions. Untreated fresh-cut fruits were used as control. The best sanitizing treatment for both pathogens and fruits was AEW at 200 ppm of free chlorine applied during 5 minutes.

Keywords: acidic electrolyzed water, apples, *Escherichia coli*, *Listeria innocua*, neutral electrolyzed water, oranges

Introduction

Over the last few years consumers demand for minimally processed fruits and vegetables has increased rapidly. To achieve this growing demand, many emerging food preservation techniques have been introduced and are being studied extensively. Fresh produce can be a vehicle for the transmission of bacterial, parasitic and viral pathogens responsible for human illnesses and a number of reports refer to raw vegetables harbouring potential foodborne pathogens (Beuchat 1996). Chlorine is the most common disinfectant used in fresh-cut industry and it is, normally, applied at a concentration of 50-200 ppm with a contact time of 1-2 min (Beuchat 1998; Abadias *et al.* 2008). Its biocidal activity depends on the amount of free available chlorine in the water that comes into contact with the microorganisms.

In recent years, acidic electrolyzed water (AEW) and neutral electrolyzed water (NEW) have been considered for application as sanitizers. These solutions can be used in food industry to reduce or eliminate bacterial populations on food products, food-processing surfaces and non-food contact surfaces (Hricova *et al.* 2008; Huang *et al.* 2008).

In Japan, the Health, Labor and Welfare Ministry has approved EW as a food additive (Yoshida *et al.* 2004). EW generators have been also approved to be used in the food industry by the U.S. Environmental Protection Agency (Park *et al.* 2002). One of the main advantages of using electrolyzed oxidizing water for disinfection is that it is produced using pure water with no added chemicals except sodium chloride (NaCl). For that reason, it has less adverse impact on environment (Kim *et al.* 2000) and it has no harm to the human body (Mori *et al.* 1997).

The purpose of this article is to review issues related to electrolyzed water in fresh cut produce and report a study undertaken in our laboratory to evaluate the disinfection efficacy of AEW and NEW in fresh-cut apples and oranges.

AEW and NEW Generation and Characteristics

There are many different types of electrolysis equipment used to produce EW but, in general, they can be divided into those that have a diaphragm and produce AEW and NEW and those that do not have a diaphragm and produce only NEW (Hricova *et al.* 2008). In equipment that produces both AEW and NEW, EW is generated by electrolysis of a diluted NaCl solution in an electrolysis cell where anode and cathode electrodes are separated by a non selective membrane (Kim *et al.* 2000). AEW is produced by the anode and it has a strong bactericidal effect on most known pathogenic bacteria due to its low pH (2-4), its high oxidation-reduction potential (ORP >1000 mV) and its content on active oxidizers like hypochlorous acid (Kim *et al.* 2000; Len *et al*. 2000). It is effective in killing food-borne pathogens *in vitro* conditions and in reducing microbial counts and pathogens in vegetables. NEW is generated like AEW, but part of the product formed at the anode is redirected into the cathode chamber. In the case of a neutral solution (pH 8) the main biocidal agents are HOCl, ClO^-, HO_2 and O_2). Because of its neutral pH, NEW is not as aggressive as AEW to the corrosion of equipment or to skin irritation, and is more stable, as chlorine loss is significantly reduced at pH 6-9 (Len *et al.* 2002).

The physical properties and chemical composition of EW vary as they depend on the concentration of NaCl, amperage level, time of electrolysis, or water flow rate (Kiura *et al*. 2002). AEW and NEW used in our laboratory were produced by an EW generator (Envirolyte EL-400, Envirolyte Industries International Ltd., Estonia). A saturated sodium chloride solution was pumped into the equipment and the current passing through the EW generator was set at 20-23 A. The properties of the treatment solutions obtained are shown on Table 1. The sodium hypochlorite solutions were more alkaline than the NEW and AEW solutions. The ORP values were also inferior which indicated that it contained less oxidizers than NEW and AEW solutions. AEW solutions had the ORP higher than NEW and sodium hypochlorite solutions.

By diluting AEW, its pH slightly increases and ORP shows the opposite behaviour. AEW200 has the lowest pH and higher ORP while the most diluted solution, AEW10, has the highest pH and the lowest ORP. When NEW solutions are diluted, both pH and ORP decreases. This way, NEW200 shows superior pH and ORP than NEW100 and NEW10. However, in the case of sodium hypochlorite solutions (SH), dilution decreases the pH and increases ORP values.

Table 1. Physicochemical properties of tested solutions.

Treatment solution	pH	ORP (mV)	Free chlorine concentration (ppm)
AEW200	2.91±0.02	1131±2	201±1.2
AEW100	3.16±0.04	1097±1	101±2.3
AEW10	3.28±0.02	1072±2	10±1.2
NEW200	7.77±0.02	774±5	199±1.5
NEW100	7.71±0.01	743±2	99±1.2
NEW10	7.64±0.01	738±2	10±0.1
SH200	11.12±0.02	571±1	200±2.4
SH100	10.76±0.01	589±1	102±1.8
SH10	9.97±0.03	598±4	10±0.1

Antimicrobial activity

Antimicrobial activity of EW can be influenced by many factors such as type of EW, exposure time, treatment temperature, pH, ORP and amperage or voltage. Additionally, different microorganisms can be more or less sensitive to the effect of EW.

Several studies have revealed that EW is effective in reducing or eliminating pathogenic microorganisms on minimally-processed vegetables (Abadias *et al.* 2008). Izumi (1999) has demonstrated that electrolyzed oxidizing water can be used for disinfecting fresh-cut carrots, bell peppers, spinach, Japanese radish and potatoes. However, its efficacy on fresh-cut fruits as only been reported on fresh-cut apples by Wang (2006).

Assays carried out in our lab were made to determine the effectiveness of AEW and NEW at 200, 100 and 10 ppm of free chlorine, in killing *Listeria innocua* on fresh-cut apples and *Escherichia coli* on fresh-cut oranges. These treatments were studied in two different washing times, 3 and 5 min. The effectiveness of those solutions was compared with distilled water and sodium hypochlorite solutions at the same chlorine concentrations and during the same periods of time.

Reduction values of *L. innocua* are shown in Table 2 and of *E. coli* in Table 3. In general, for both pathogens, exposure time of 5 min allowed a higher microbial reduction than 3 min. The reduction of *L. innocua* was higher in apples than in oranges, but for both fruits the highest reduction values were achieved with AEW200 treatment (1.70 and 1.10 log cfu g^{-1}). The reduction of *L. innocua* with AEW100 was similar to that obtained with the other sanitizer solutions containing 200 ppm of free chlorine, indicating that the application of AEW, during 5 min, allowed the use of treatments with half concentration of chlorine.

In relation to the population of *E. coli,* the highest reduction was also observed in fresh-cut fruits washed with AEW during 5 min (2.1 log cfu g^{-1}), followed by washes with AEW100 for 3 min and NEW200 for 5 min.

Table 2. Reduction of *L. innocua* population on 1 g of fresh-cut apples and oranges treated during 3 or 5 min with the different disinfection solutions.

Treatment	Time (min)	Reduction (log cfu g^{-1}) in fresh-cut apples[a]	Reduction (log cfu g^{-1}) in fresh-cut oranges[a]
AEW200	3	1.37 bc	0.55 cd
	5	1.70 a	1.10 a
AEW100	3	1.16 cd	0.65 c
	5	1.30 bc	0.72 c
AEW10	3	0.71e	0.29 e
	5	0.81e	0.36 de
NEW200	3	1.26 cd	0.66 c
	5	1.12 cd	0.99 b
NEW100	3	0.80 e	0.45 d
	5	1.08 d	0.65 c
NEW10	3	0.69 e	0.15 f
	5	0.69 e	0.35 de
SH200	3	1.16 cd	0.65 c
	5	1.50 b	0.77 c
SH100	3	1.25 cd	0.35 de
	5	1.11 d	0.59 cd
SH10	3	0.81 e	0.20 f
	5	0.62 e	0.15 f
DW	3	0.72 e	0.21 f
	5	0.78 e	0.25 ef

[a]Values are the mean of 3 experiments with 3 replicates each. Within type of fruit, rows with different letters indicate significant difference between means using LSD (P<0.05%).
Initial counts of *L. innocua* were 1.2×10^{6} cfu g^{-1} on fresh-cut apples and 4.5×10^{5} cfu g^{-1} on fresh cut oranges.

Table 3. Reduction of *E. coli* population on 1 g of fresh-cut oranges treated during 3 or 5 min with the different disinfection solutions.

Treatment	Time (min)	Reduction (log cfu g^{-1}) in fresh-cut oranges[a]
AEW200	3	1.80 ab
	5	2.05 a
AEW100	3	1.11 d
	5	1.36 c
AEW10	3	0.90 e
	5	0.87 e
NEW200	3	1.31 c
	5	1.70 b
NEW100	3	1.34 c
	5	1.56 bc
NEW10	3	0.85 e
	5	0.93 e
SH200	3	1.20 cd
	5	1.45 bc
SH100	3	1.44 c
	5	1.48 c
SH10	3	0.90 e
	5	0.99 e
DW	3	0.70 e
	5	0.85 e

[a]Values are the mean of 3 experiments with 3 replicates each. Different letters indicate significant difference between means using LSD ($P<0.05\%$).
Initial counts of *E. coli* on fresh-cut oranges were 2.6×10^{5} cfu g^{-1}

For both pathogens and fruits, the treatments with concentrations of free chlorine of 10 ppm, resulted in microbial reductions similar to those obtained with distilled water, in almost all the tested situations.

The results obtained in our work indicate that AEW or NEW could be used as an alternative sanitizer to sodium hypochlorite. Park *et al.* (2004) has demonstrated that EW water is very effective for inactivating *E.* O157:H7 and *L. monocytogenes* in a wide pH range (between 2.6 and 7.0), if sufficient free chlorine (>2 ppm) is present. In the assay carried out by Abadias *et al.* (2008), NEW with 89 ppm of free chlorine reduced the bacterial population inoculated on lettuce by 1.2 to 1.5 log cfu g^{-1} and 1.4 to 1.7 log cfu g^{-1} for 1 and 3 min treatments, respectively. Park *et al.* (2001) reported that when lettuce was immersed in EW with 45 ppm of free chlorine for 1 min, populations of *E. coli* O157:H7 and *L. monocytogenes* were reduced 2.8 and 2.4 log cfu $leaf^{-1}$, respectively. Koseki (2004) showed that AEW treatment on strawberries, for 10 min, resulted in a reduction of naturally present aerobic bacteria by 1.6 log cfu g^{-1} and coliforms by 2.4 log cfu g^{-1}.

The results obtained during the present work, showed that the higher microbial reductions were achieved when the tested fruits (apple and orange) were treated with AEW, during 5 min, regardless of the chlorine concentrations.

The reduction values obtained with AEW and NEW at 200 and 100 ppm of free chlorine ranged, for *L. innocua,* from 0.7 to 1.70 log cfu g^{-1} on apples and 0.5 to 1.1 log cfu g^{-1} on oranges. For *E. coli* the reductions obtained varied from 0.5 to 1.7 log cfu g^{-1} on oranges. Studies with *E. coli* carried out in our laboratory allowed reductions in other fruits, such apple (Nunes *et al.* 2010) and pears (data not shown), of more than 2.4 log cfu g^{-1} using AEW at 100 ppm of free chlorine during 5 min. These results were also achieved in other works. Pre-cut produces, treated with electrolyzed oxidizing water by dipping, rinsing or dipping/blowing, showed a bacterial reduction of 0.6-2.6 log cfu g^{-1} (Izumi 1999). Yang *et al.* (2003) reported that fresh-cut lettuce dipped in electrolyzed oxidizing water (pH 7) with 300 ppm of free chlorine, for 5 min, could reduce 2 log cfu g^{-1} of *Salmonella typhimurium*, *E. coli* O157:H7 and *L. monocytogenes* populations. Results presented by other authors have shown that both electrolyzed oxidizing water containing 200 and 444 ppm of free chlorine significantly reduces the populations of *E. coli* O157:H7, *S. enteritidis* and *L. monocytogenes* on the surfaces of tomatoes without affecting their sensory quality (Bari *et al.* 2003; Deza *et al.* 2003).

The disinfection washing time may differ depending on the treatment solution and on the type of microorganisms present on the fruits. Wang *et al.* (2007) reported that the microbial population reduction achieved with different sanitizers, including electrolyzed water, was higher for contact periods of 5 min than for exposures of 1 or 3 min. On the other hand, some studies revealed that in the case of vegetables, the increasing of washing time above 1 or 2 min had no effect on bactericidal activity (Adams *et al.* 1989; Beuchat *et al.* 1998). In an assay made by Koseki *et al.* (2000) with AEW treatment on lettuce, the length of treatment time did not increase the reduction of aerobic bacteria population. Park *et al.* (2001) found no significant differences in efficacy based on treatment time when treating lettuce with EW. Abadias *et al*. (2008) also reported that different washing times of 1, 3 and 5 min with NEW or with SH does not affect significantly antimicrobial activity on different fresh-cut vegetables. This difference could be due to the fact that reduction in microbial populations on fresh-cut produce is dependent upon the type of produce (FDA 2001).

Chlorine reacts with organic matter and some components from tissues of cut produce surfaces, such as apple juice, could neutralize some of the chlorine before it reaches microbial cells, reducing its effectiveness which does not occur in vegetables (specially in non cut surfaces). Subsequently, increasing the time of exposure increases the antimicrobial activity in apple slices. Chlorine form present in AEW (HOCl) is more stable than in SH (ClO^-), thus chlorine form present in SH solutions reacts quickly and this could be an explanation why increasing washing time does not increase the reduction of the microorganism population, in washings with SH. Anonymous (1997) reported that HOCL is 80 times more effective as a sanitizer than an equivalent concentration of the hypochlorite ion (ClO^-).

Labbé and Garcia (2001) reported that a longer exposure time is necessary to eliminate microorganisms harboured in porous surfaces (as apple tissues) than in nonporous surfaces. Wang *et al*. (2009) reported that different times are required to achieve the same level of inactivation of *E. coli* in fresh-cut cantaloupe melon and in fresh-cut apple. This reduction has a dual-phasic behaviour with a first fast inactivation phase and a much lower inactivation in a second phase, due to a different accessibility of the sanitizer agents to pathogens. As a result, during the first phase, the loosely attached cells on the superficial areas will be quickly removed when compared to the second phase removal.

Conclusions

The possibility of using AEW or NEW instead of chlorine solutions in the fresh-cut industry may help to improve the safety of the products and workers. Using electrolyzed water in the fresh-cut industry has the advantages of lowering adverse impact on the environment and human health, as no hazard chemicals are added during processing. In addition, it is less expensive than other sanitizing techniques because the only expenses are water, sodium chloride and energy.

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