Use of Electrolyzed Oxidizing Water for Quality Improvement of Frozen Shrimp

MIN HUI LOI-BRADEN, TUNG-SHI HUANG, JANG-H KIM, CHENG-I WEI, AND JEAN WEESE

ABSTRACT: The bactericidal effect of electrolyzed oxidizing (EO) water was evaluated on *Escherichia coli* O157:H7-inoculated and *Salmonella*-inoculated shrimp. The shrimp were inoculated on day 0 and stored frozen at –20 °C. Bacterial enumeration was done on days 0, 24, 49, and 119 of frozen storage. Acidic EO water at 40 ppm free available chlorine was as effective as aqueous chlorine of the same concentration and was significantly more effective (P < 0.05) than tap water in reducing pathogen load on the inoculated shrimp. Further reduction of pathogen numbers was observed after each frozen storage period. Prewashing with alkaline EO water did not enhance the bactericidal activity of the acidic EO water on the shrimp. The washed acidic EO water of the inoculated shrimp had a nondetectable bacterial population compared with treated aqueous chlorine, alkaline EO water, and tap water. Non-inoculated shrimp subjected to similar treatments were served cooked or uncooked to a minimum of 10 experienced panelists for sensory evaluation on days 0, 24, 49, and 119 of frozen storage. The cooked shrimp were evaluated for the presence of off-odor, juiciness, tenderness, shrimpy flavor, aftertaste, and overall acceptability; whereas the raw shrimp were evaluated for color, firmness, presence of off-odors, melanosis, and overall acceptability. Raw shrimp thawed from each frozen storage period were stored at refrigeration temperature (4 °C) for 3 d to observe for melanosis. No difference of sensory attributes was detected among the various treatment groups. Therefore, acidic EO water can be used as an effective disinfectant to replace aqueous chlorine for thawing shrimp blocks.

Keywords: EO water, aqueous chlorine, shrimp, bactericidal, and sensory

Introduction

It is estimated by the Centers for Disease Control and Prevention (CDC) that foodborne pathogens cause approximately 76 million cases of illness, 325,000 hospitalizations, and 5,000 deaths in the United States each year (Mead and others 1999). Microbial contamination can occur at any of the multiple stages of food preparation, from production and harvest to processing to serving the food. To minimize and prevent foodborne diseases, it is necessary to prevent initial contamination and eliminate further amplification of these pathogens.

Aqueous chlorine has long been known for its bactericidal activity. It is widely used in food processing plants for control of microbial growth (Eifert and Sanglay 2002). However, aqueous chlorine has limitations for use as an effective sanitizer. The bactericidal activity of aqueous chlorine is decreased at alkaline pH, leading to the need for much higher chlorine content to achieve the desired bactericidal effect (Len and others 2000). Aqueous chlorine needs huge amounts of space for storage and can be hazardous when stored in large amounts. Therefore, it is important to explore alternative disinfectants for use in the food industry.

Electrolyzed oxidizing (EO) water has been proven to possess strong bactericidal activity against various foodborne pathogens (Venkitanarayanan and others 1999; Kim and others 2000a; Park and others 2002). EO water can be easily and conveniently generated on-site from a very dilute sodium chloride (NaCl) solution by using a commercial electrolytic cell containing a positively charged anode and a negatively charged cathode separated by a membrane. NaCl undergoes electrolysis to produce acidic hypochlorous acid (HOCl) and basic sodium hydroxide (NaOH) according to the following equation:

\[
\text{NaCl} + \text{H}_2\text{O} \rightarrow \text{NaOH} + \text{HOCl}
\]

The acidic EO water has a bactericidal effect, and alkaline EO water has a cleaning function. The concentration of HOCl depends on the concentration of NaCl used, as well as the amperage and voltage of the electrolytic cell (Len and others 2000; Hsu 2003).

Because seafood is highly perishable, it is not uncommon for the seafood industry to use aqueous chlorine for washing seafood to meet microbiological standards, prevent spoilage, and increase product shelf life (Huss 1994). Imported frozen shrimp blocks are often thawed in seafood processing plants using chlorinated water. They are then repacked according to sizes, frozen again, and distributed to local retailers. The strong bactericidal activity of EO water can be used to replace aqueous chlorine for thawing frozen shrimp blocks.

Although much research had been done with EO water on fresh produce, meats, and poultry, limited work is done with seafood, particularly shrimp. The objectives of this study were to determine (1) the bactericidal activity of EO water compared with aqueous chlorine, against *Salmonella* and *Escherichia coli* O157:H7 spiked on the shrimp, (2) whether prewashing of the shrimp with alkaline EO water improves the bactericidal activity of acidic EO water, (3) whether shrimp treated with EO water followed by frozen storage have lower bacterial count than shrimp treated with aqueous chlorine and tap water, and (4) whether treatment with EO water or aqueous chlorine followed by frozen storage affects the sensory and organoleptic properties of shrimp.
Materials and Methods

Preparation of treatment and working solutions
A 15% NaCl solution (NaCl, Sigma Laboratories, St. Louis, Mo., U.S.A.) in deionized water was used to generate EO water with an EO water generator (ROX 20 TA, Hoshizaki Electric, Shamane, Japan). The EO water generator was set at 14 amperes and 10 volts to produce approximately 40 ppm residual chlorine in acidic EO water. After the generator had reached a stabilized reading for the desired amperage and voltage settings (about 15 min), alkaline EO water was collected from the cathode side of the generator and acidic EO water collected from the anode side. Both alkaline and acidic EO water were used within 5 min upon collection. A 40 ppm aqueous chlorine solution was prepared from a sodium hypochlorite solution (NaOCl, Clorox®, Clorox, Co., Oakland, Calif., U.S.A.) with deionized water. The aqueous chlorine solution was stored in a dark brown flask and used within 5 min of preparation. Tap water was used as the control for the experiment. To avoid accumulation of inorganic compounds from the plumbing line, the faucet was left on continuously and tap water was collected when needed.

Oxidation-reduction potential (ORP) and pH measurements were taken for all 4 solutions immediately before and after treatment with shrimp samples using a dual-scale pH meter (Accumet Model 15, Fisher Scientific Co., Suwanee, Ga., U.S.A.) with ORP and pH electrodes, respectively. Total available chlorine (TAC) was determined for acidic EO water, alkaline EO water, aqueous chlorine solution, and tap water immediately before and after treatment with shrimp sample using iodometric titration (APHA 1999). N, N-diethyl-P-phenylenediamine (DPD) ferrous titrimetric method was used for chlorine species differentiation (APHA 1999).

Preparation of bacterial suspension for inoculation
Three strains of nalidixic acid and novobiocin resistant E. coli O157:H7 (505B, 204P, and 301C) were obtained from the Center for Food Safety, Univ. of Georgia (Griffin, Ga., U.S.A.). Each strain was maintained separately on trypticase soy agar (TSA, Difco Laboratories, Detroit, Mich., U.S.A.) containing 0.02% nalidixic acid (C₁₀H₁₁N₂O₃, Sigma-Aldrich Co.) and 0.025 ppm novobiocin (C₁₁H₁₀N₄O₅Na, Sigma Chemical Co.). Additionally, 3 strains of nalidixic acid–resistant Salmonella enterica serotypes Enteritidis, Typhimurium, and Mission obtained from N. A. Cox (Athens, Ga., U.S.A.) were similarly maintained on TSA containing 0.02% nalidixic acid. The TSA plates were stored at 4°C.

One day before each experiment, a single colony of each bacterial strain was transferred into 15 mL of trypticase soy broth (TSB, Difco Laboratories) and incubated in a gyrotory water bath shaker (New Brunswick Scientific Co., Inc., Edison, N.J., U.S.A.) at 37°C and 100 rpm for 14 h. On the day of the experiment, 3 mL of each culture broth was removed and pooled in a sterile centrifuge tube to obtain a 3-serotype mixture, and centrifuged (IEC Clinical Centrifuge, ThermoeIC Cooperation, Needham, Mass., U.S.A.) at 1500 g for 10 min. The bacterial mixture was washed twice with 5 mL of Butterfield’s phosphate buffer. The final bacterial pellet was resuspended in 18 mL of Butterfield’s phosphate buffer and served as bacterial stock solution.

Absorbance reading was measured at 640 nm with a spectrophotometer (DU 7400, Beckman Coulter Inc., Fullerton, Calif., U.S.A.). The number of E. coli O157:H7 and Salmonella were estimated using standard curves. Preliminary studies showed that bacterial suspensions prepared as described previously routinely yielded a concentration within the range of 10⁶ colony-forming units (CFU)/mL for E. coli O157:H7 and 10⁹ CFU/mL for Salmonella. Both bacterial stock solutions were further diluted using Butterfield’s phosphate buffer to yield a 10⁷ CFU/mL suspension for inoculation of the shrimp sample. The concentration for both bacterial suspensions was enumerated using the spread method on TSA plates containing corresponding antibiotics for E. coli O157:H7 and Salmonella. Bacterial colonies on these plates were counted after incubation at 37°C for 24 h.

Preparation and inoculation of shrimp sample
Brown shrimp (Farfantepenaeus aztecus, 30 to 40 count) was obtained from Southern Fish and Oyster Co. (Mobile, Ala., U.S.A.). The shrimp were harvested by trawler in the Gulf of Mexico the night before and transported in ice to Auburn in an ice chest. The heads were removed, and all shrimp were stored at −20°C. One day before the experiment, the frozen shrimp were transferred into a refrigerator (4°C) for slow thawing.

Shrimp (13 ± 3 g/shrimp) were immersed in a 3-strain bacterial suspension (1 g shrimp : 5 mL bacterial suspension) for 2 min for inoculation. Excess bacterial suspension was allowed to drain completely, and the shrimp were placed in a biosafety hood for 10 min at room temperature (23 ± 2°C) to allow for bacterial attachment.

Four sets of spiked shrimp (25 g each) were each homogenized at high speed in sterile blender (Blend Master, Hamilton Beach, Washington, N.C., U.S.A.) with 225 mL of Butterfield’s phosphate buffer. After serial dilution of the homogenates with Butterfield’s phosphate buffer, 0.1 mL aliquot of each dilution was plated on quadruplicate TSA plates infused with corresponding antibiotic for E. coli O157:H7 and Salmonella spp. The plates were incubated at 37°C for 24 h, and bacterial colonies were recorded. Non-spiked shrimp were processed using the same procedures and plated on TSA plates without antibiotics for enumeration of normal flora.

Treatment of shrimp and bacteriological analysis
The inoculated shrimp (400 g) were soaked with continuous stirring at 45 rpm in 1.5 L treatment solutions for the following time periods under the room temperature (22°C): tap water for 10 min, alkaline EO water for 5 min followed by acidic EO water (40 ppm) for 2 min, alkaline EO water for 5 min followed by acidic EO water (40 ppm) for 5 min, or aqueous chlorine (40 ppm) for 10 min. At the end of each treatment, 4 sets of shrimp samples (approximately 25 g each) were transferred to sterilized blenders and homogenized in 225 mL of Butterfield’s phosphate buffer.

The bacterial colonies were isolated and recorded using the same methods mentioned in the previous section. The remaining shrimp were divided into 25-g portions and stored separately in quart-sized Ziploc® bags at −20°C for 24, 49, and 119 d for bacterial enumeration. The inoculated shrimp that received no washing treatment with any of the test solution were also enumerated for E. coli O157:H7 and Salmonella in a similar manner for background check. The experiment was replicated twice.

On each test day, 4 bags were removed from each treatment group, homogenized in 225 mL of Butterfield’s phosphate buffer, and processed as described previously to enumerate bacterial loads on the test shrimp. The spiked shrimp that received no washing treatment were also tested at each storage time.

Bacteriological analysis of treatment solutions
Residual bacteria in all treatment solutions were determined by serially diluting the solutions in Butterfield’s phosphate buffer and using 0.1 mL aliquot of the diluted samples for spread plating onto TSA plates containing the appropriate antibiotics. Colonies were counted after incubation at 37°C for 24 h.

To further confirm the effectiveness of EO water as a bactericide, a 25-mL aliquot of all wash solutions was added into 225 mL of half-strength tryptic soy broth for pre-enrichment. The broth was incubated at 37°C for 6 h in a gyrotory water bath shaker at 100
Shrimp washing using electrolyzed oxidizing water . . .

Sensory evaluation of the shrimp

Shrimp that were not inoculated with bacteria were used for sensory evaluation using a minimum of 10-member experienced panel. The panel members were recruited from the Dept. of Nutrition and Food Science, Auburn Univ. Recruitment of panel members was based on being (1) experienced from prior participation in sensory tests, (2) no allergic reactions to eating shrimp, (3) a shrimp eater, and (4) willing to take part in all sensory tests conducted. The sensory panel was asked to evaluate changes in sensory attributes among the various treatment shrimp groups that had been stored at −20 °C for 24, 49, and 119 d.

Shrimp were similarly processed with different washing solutions. On day 0 of the experiment, the 1st group of shrimp were peeled and prepared by boiling in water for 2 min. The samples were chilled for 12 h at 4 °C and served cold to sensory panel members. A random 3-digit code was used for designating the samples, and chilled apple juice was provided for palate cleansing between shrimp samples. The cooked shrimp was evaluated for off-odors, juiciness, tenderness, shrimpmy flavor, aftertaste, and overall acceptability. After sensory tests, panel members were asked to evaluate visual and physical characteristics of raw shrimp that had undergone similar treatments. Ten shrimp from each treatment group were placed on a white paper plate and a 3-digit random number was used to designate each treatment group. In addition to the treatment groups, 10 shrimp that have not undergone any form of treatment were displayed along with the treatment samples to serve as a control group for comparison. Panel members were asked to evaluate the raw shrimp for color, firmness, off-odors, black spot formation, and overall acceptability.

All evaluations were recorded by placing a vertical mark for each sample along a nongraduated horizontal line for each attribute (Lawless and Heyman 1999). After the panelists made their evaluations, a 10-point scale was applied to the nongraduated line. Score 1 was assigned to attributes that were considered most inferior whereas score 10 was assigned to attributes that were considered most superior. All evaluations were performed in an environmentally controlled sensory partitioned booth and under yellow light furnished by a recessed 60 W bulb. Both sensory and physical tests were done on the same day and repeated twice with each set of sessions separated by a 3-h recess.

In an accompanying test, 10 raw shrimp from each treatment group were stored at 4 °C for 72 h and then examined for the occurrence of black spots on each shrimp. Numerical scores from 1 to 5 were given to each shrimp, with 1 being least severe in formation of black spots and 5 being most severe.

These described procedures were repeated on shrimp stored at −20 °C for 24, 49, and 119 d to determine the effect of frozen storage on sensory changes.

Statistical analysis

The bacterial counts on the shrimp samples and wash solutions were transformed to units of Log_{10} CFU/g or Log_{10} CFU/mL. Data were analyzed using analysis of variance (ANOVA) and Tukey’s test for comparison of means between pairs. Scores from the sensory tests were analyzed using general linear models procedures and Tukey’s test for comparison of means between pairs (SAS Inst., Inc., 1996).

Results and Discussion

Chlorine species, pH, and oxidation-reduction potential of the EO water, aqueous chlorine, and tap water

Table 1 shows changes of the contents of TAC and readings of pH and ORP of various test solutions before and after washing of shrimp samples. The freshly prepared acidic EO water and aqueous chlorine had approximately 40 ppm of TAC; the tap water had very small amount of TAC; and the alkaline EO water had none. The TAC content of the acidic EO water and aqueous chlorine was reduced after treatment of the shrimp due to reactions of free chlorine with the shrimp constituents to form various reaction products (Wei and others 1985; McPherson 1993).

Washing of the shrimp with tap water, acidic EO water, or alkaline EO water caused slight changes of the pH readings of these washing solutions (Table 1). However, the pH of the aqueous chlorine was reduced from 8.70 to 7.87 after washing of the shrimp. Similar result was also observed in the treated aqueous chlorine after washing of chicken meat or beef (West and others 2001).

Except the alkaline EO water, washing of shrimp caused significant reduction of the ORP values of the treated tap water, aqueous chlorine, and acidic EO water (Table 1). Park and others (2002) also reported similar findings about the reduction of ORP values for the treated acidic EO water and chlorinated water after washing of chicken wings. The reduction of ORP in these treated solutions was related to the loss of oxidants, including free available chlorine, due to their interaction with the organic matters. Park and others (2004) found that the ORP value of acidic EO water increased with increasing chlorine concentration. The high ORP in the acidic EO water could cause modification of bacterial metabolic fluxes and ATP production, probably due to the change in the electron flow in the cells (Park and others 2002).

DPD ferrous titration was used to determine changes of various chlorine species in the washing solutions before and after treatment of shrimp samples. The high contents of free available chlorine (FAC) in the acidic EO water and aqueous chlorine were dramatically deplet-
Shrimp washing using electrolyzed oxidizing water... ed after washing (Table 2). This reduction of FAC contents was accom-
panied by the formation and increases of the contents of monochlo-
romines (NH₂Cl) in the treated solutions due to interaction of the
shrimp organic matters with FAC (Table 2). The washing of shrimp did
not cause the formation of dichloroamines or change the min content
of chlorine dioxide in the acidic EO water and aqueous chlorine (Table
2). Because the alkaline EO water had no chlorine content, this water
together with its treated solution after washing of the shrimp were not
subject to DPD ferrous titration. The types of chloramines formed
after washing of the shrimp depend on the pH, temperature, contact
time, and the initial chlorine to organic matter ratio (White 1999).

### Changes of bacterial loads on the shrimp after treatment with test solutions and storage at −20 °C for various time periods

The brown shrimp used in this study had an endogenous microf-
lora population of 3.72 × 10⁴ CFU/g to 6.92 × 10⁴ CFU/g. The bacte-
rrial suspensions used to inoculate the shrimp had a concentration of
1.45 × 10⁶ CFU/mL for E. coli O157:H7 and 4.88 × 10⁶ CFU/mL for
Salmonella spp. After inoculation, the inocula on the shrimp samples were analyzed, and the shrimp had a bacterial load of 5.57
× 10⁵ CFU/g (5.75 log CFU/g) for E. coli O157:H7 and 2.28 × 10⁶
CFU/g (6.36 log CFU/g) for Salmonella spp., respectively.

Washing of the inoculated shrimp with tap water, aqueous chlorine,
or electrolyzed oxidizing (EO) water and then stored at –20 °C for various time periods (Waterman and Small 1998).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 0</th>
<th>Day 24</th>
<th>Day 49</th>
<th>Day 119</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculated control</td>
<td>5.70±0.22a</td>
<td>4.21±0.10c</td>
<td>4.27±0.04c</td>
<td>3.96±0.12c</td>
</tr>
<tr>
<td>Tap water&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.05±0.22b</td>
<td>3.11±0.25d</td>
<td>3.38±0.19d</td>
<td>2.79±0.29d</td>
</tr>
<tr>
<td>Aqueous chlorine&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.97±0.09b</td>
<td>3.09±0.09d</td>
<td>3.02±0.24e</td>
<td>2.31±0.29d</td>
</tr>
<tr>
<td>EO water (2 min)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.00±0.10b</td>
<td>3.09±0.09d</td>
<td>3.02±0.24e</td>
<td>2.31±0.29d</td>
</tr>
<tr>
<td>EO water (5 min)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.08±0.27b</td>
<td>3.10±0.17d</td>
<td>3.03±0.14e</td>
<td>2.41±0.38d</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are means ± standard deviations from quadruplicate samples in each experiment. CFU = colony-forming unit.
<sup>b</sup>Data in the same column with different superscripts are significantly different (P < 0.05).
<sup>c</sup>Tap water for 10 min.
<sup>d</sup>Aqueous chlorine for 10 min.
<sup>e</sup>Alkaline EO water for 5 min followed by acidic EO water for 2 min.
<sup>f</sup>Alkaline EO water for 5 min followed by acidic EO water for 5 min.

This finding is consistent with the results of Park and others (2002) that acidic EO water and aqueous chlorine had no differ-
effectiveness in reducing Campylobacter jejuni on the chicken wings. They also found no difference between the 10- and 30-min treatments with the acidic EO water in reducing the bacterial numbers. Similarly, we noticed in this study that washing of the inoculated shrimp with acidic EO water for 2 or 5 min did not differ in effectiveness in reducing the number of residual pathogens (Table 3). The complex nature of the shrimp or chicken wing to limit the penetration of the disinfectant could contribute to there being no difference in bactericidal activity between the 2 washing solutions or between the 2 treatment times with the acidic EO water. The complex surface of the shrimp also provides a microenvironment to allow bacterial entrapment and survival in the folds, crevices, and pores. The presence of proteins, fatty acids, and oils could help shield bacterial cells from external stresses such as the bactericidal activity of the chlorine washing solu-
tions (Waterman and Small 1998).

Because of its emulsifying capability, sodium hydroxide is often used as a cleaning agent to remove oils. However, pre-washing with alkaline EO water of the inoculated shrimp did not enhance the bactericidal activity of the acidic EO water in the test system. Appar-
tently, such pre-washing treatment with alkaline EO water did not improve the environment of the inoculated shrimp for better penetration of the acidic EO water to exert its bactericidal activity.

The prolonged frozen storage at −20 °C caused dramatic reduction in the populations of E. coli O157:H7 and Salmonella spp. on the treated shrimp. The data in Table 3 showed that on each test day of frozen storage, the inoculated shrimp that received washing with tap water, aqueous chlorine, or EO water before frozen storage had a significantly
Shrimp washing using electrolyzed oxidizing water...

lower ($P < 0.05$) number of *E. coli* O157:H7 or *Salmonella* spp. than the inoculated control. However, there was generally no difference in bacterial loads among the 4 treatment groups at all test periods except for the 49-d samples inoculated with *E. coli* O157:H7, in which the shrimp treated with aqueous chlorine or EO water for 2 or 5 min had significantly lower bacterial counts than the tap water treatment group.

A comparison of time-related changes of bacterial loads on frozen shrimp showed a steep decline of the number of *E. coli* O157:H7 and *Salmonella* spp. in the 1st 24 d of storage. The treated shrimp then showed a gradual decline in bacterial numbers from day 24 to day 119 of frozen storage. Restaino and others (2001) found that inflicted injury of *E. coli* O157:H7 in beef infusion was maximal after storage at –25 °C for 30 d, with most rapid cellular death and injury occurring in the 1st 10 d. Apparently, the pores and crevices of the shrimp as well as the presence of shrimp proteins and fatty acids provide a better protective environment for the injured bacterial pathogens to cope with the cold stress of frozen storage.

**Residual bacterial populations in treated tap water, aqueous chlorine, and EO water**

The populations of *E. coli* O157:H7 and *Salmonella* spp. in various washing solutions following treatment of the inoculated shrimp are displayed in Table 4. The identity of the bacterial species in the treated solutions was confirmed by using the API 20E biochemical test kit (data not shown). As expected, with hardly any bactericidal activity existed, the washed tap water had the highest residual bacterial population for both *E. coli* O157:H7 and *Salmonella* spp.

The treated aqueous chlorine and alkaline EO water had significantly lower ($P < 0.05$) bacterial populations than those of the treated tap water. The less effective bactericidal effect of the treated aqueous chlorine was related to the prevalence of the less bactericidal species of *OCl* in the test solution. At pH 8.70, the HOCl in aqueous chlorine which was prepared from Cloroxy™ (NaOCl) was dissociated to *OCl* (Len and others 2000) to exert less bactericidal activity. The aqueous chlorine that Kim and others (2000a, 2000b) prepared by dissolving Cl₂ gas into water at pH 3.9 had about 10 ppm of *FAC* existing as HOCl. This aqueous chlorine solution had a similar effective bactericidal activity as the acidic EO water against *E. coli* O157:H7 in pure culture.

The alkaline EO water had a significantly ($P < 0.05$) better bactericidal activity than aqueous chlorine in killing *E. coli* O157:H7 and *Salmonella* spp. (Table 4). Sodium hydroxide is a common disinfectant used in many food processing plants, primarily for cleaning of facilities required to disinfect the wastewater generated from the washing of various food systems with acidic EO water. Such wastewater can be discarded directly into the municipal sewage system.

**Sensory evaluation of the washed raw and cooked shrimp**

The sensory evaluation panel detected no difference ($P > 0.05$) among the 4 treatment groups for each of the quality attributes of raw shrimp (color, firmness, odor, black spot, and overall acceptability) (Table 5). The panel members also detected no difference among the 4 cooked shrimp groups for each of the quality attributes (odor, juiciness, tenderness, shrimpmy flavor, aftertaste, and overall acceptability) (Table 5). Except for shrimpmy flavor, all the sensory attributes with the cooked shrimp had scores greater than 5.43. The inferior scores associated with the shrimpmy flavor attribute of the cooked shrimp could be due to the detection of chlorine residues in shrimp tissues by the panelists. Johnson and others (1983) found that shrimp immersed in 150 ppm HOCl solution for 30 min led to detection of off-flavor in 75% of the edible shrimp. Approximately 2% of the chlorine was incorporated into shrimp tissues.

On each of the prolonged storage days, days 24, 49, or 119, the sensory evaluation panel detected no difference among the 4 treatment groups for each of the quality attributes of raw shrimp (color, firmness, odor, black spot and overall acceptability) (data not shown). All the raw shrimp sensory attributes on each test day received similar scores as those of the freshly treated shrimp with the 4 different washing solutions. The sensory panelists also gave no different scores to the 4 groups of cooked shrimp for each of the associated quality attributes (odor, juiciness, tenderness, shrimpmy flavor, aftertaste and overall acceptability) (data not shown). Again, the scores received for each sensory attribute associated with these cooked shrimp were similar to those of the freshly cooked shrimp. As with the freshly cooked shrimp on day 0, these cooked shrimp had lower scores associated with shrimpmy flavor attribute compared with the scores for the other attributes.

Yamagata and Low (1995) showed that banana shrimp, after 3 mo of storage at –20 °C, still had very good appearance although with a slightly softer texture. They attributed this loss of shrimp texture to the possible formation of formaldehyde during frozen storage. The enzyme responsible for formaldehyde formation was demonstrated to remain active at about –29 °C. However, in this study, the loss of firmness was not detected by the sensory panel in the tested shrimp even after 119 d of frozen storage.

Table 4—Residual populations of *Escherichia coli* O157:H7 and *Salmonella* spp. (log CFU/mL) in various washing solutions following treatment of the inoculated shrimp samples

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>E. coli</em> O157:H7</th>
<th><em>Salmonella</em> spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TW (10 min)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.95 ± 0.15a</td>
<td>5.68 ± 0.05a</td>
</tr>
<tr>
<td>AC (10 min)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.20 ± 0.10b</td>
<td>5.04 ± 0.14b</td>
</tr>
<tr>
<td>Acidic EW (2 min)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Acidic EW (5 min)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Alkaline EW (5 min)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.72 ± 0.16c</td>
<td>3.38 ± 0.32c</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are mean ± standard deviations from quadruplicate samples. CFU = colony-forming unit; ND = not detectable.
<sup>b</sup>Data in the same column with different superscripts are significantly different ($P < 0.05$) from each other.
<sup>c</sup>Tap water for 10 min.
<sup>d</sup>Aqueous chlorine for 10 min.
<sup>e</sup>Acidic electrolyzed oxidizing (EO) water for 2 min.
<sup>f</sup>Acidic EO water for 5 min.
<sup>g</sup>Alkaline EO water for 5 min.

<sup>jejuni</sup>. From the point of practical implication, the washing with acidic EO water prevents cross-contamination between shrimp samples and the food processing facilities. Moreover, no further treatment would be required to disinfect the wastewater generated from the washing of various food systems with acidic EO water. Such wastewater can be discarded directly into the municipal sewage system.
Table 5—Sensory scores for raw and cooked shrimp that had been treated with tap water, aqueous chlorine, or electrolyzed oxidizing (EO) watera

<table>
<thead>
<tr>
<th></th>
<th>Raw shrimp</th>
<th></th>
<th></th>
<th>Cooked shrimp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TW²-R</td>
<td>AC²-R</td>
<td>E2²-R</td>
<td>E5²-R</td>
</tr>
<tr>
<td>Color</td>
<td>7.0 ± 1.9</td>
<td>7.0 ± 1.9</td>
<td>7.3 ± 1.0</td>
<td>7.1 ± 1.6</td>
</tr>
<tr>
<td>Firmness</td>
<td>6.2 ± 2.1</td>
<td>6.2 ± 2.2</td>
<td>4.9 ± 1.9</td>
<td>6.2 ± 1.9</td>
</tr>
<tr>
<td>Odor</td>
<td>7.4 ± 1.6</td>
<td>7.0 ± 1.9</td>
<td>7.0 ± 1.6</td>
<td>6.6 ± 1.9</td>
</tr>
<tr>
<td>Black spot</td>
<td>7.1 ± 1.4</td>
<td>6.9 ± 2.2</td>
<td>5.6 ± 2.1</td>
<td>6.2 ± 1.7</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>6.5 ± 1.6</td>
<td>5.4 ± 2.2</td>
<td>6.3 ± 1.7</td>
<td>6.6 ± 1.6</td>
</tr>
<tr>
<td>Odor</td>
<td>6.6 ± 2.0</td>
<td>6.9 ± 1.8</td>
<td>7.6 ± 1.7</td>
<td>6.8 ± 1.5</td>
</tr>
<tr>
<td>Juiciness</td>
<td>5.4 ± 2.4</td>
<td>6.2 ± 1.7</td>
<td>5.7 ± 2.3</td>
<td>5.9 ± 2.3</td>
</tr>
<tr>
<td>Tenderness</td>
<td>6.7 ± 1.9</td>
<td>6.3 ± 1.8</td>
<td>6.2 ± 2.4</td>
<td>6.7 ± 2.0</td>
</tr>
<tr>
<td>Shrimpy flavor</td>
<td>4.7 ± 2.2</td>
<td>5.1 ± 1.9</td>
<td>3.6 ± 1.7</td>
<td>4.3 ± 2.3</td>
</tr>
<tr>
<td>Aftertaste</td>
<td>7.0 ± 1.8</td>
<td>7.2 ± 1.6</td>
<td>7.9 ± 1.4</td>
<td>7.3 ± 1.6</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>6.0 ± 2.6</td>
<td>6.6 ± 1.9</td>
<td>6.2 ± 2.2</td>
<td>6.2 ± 2.2</td>
</tr>
</tbody>
</table>

aValues are means ± standard deviations from at least a 10-panelist scoring.

Although the treated shrimp that had been stored at −20 °C for up to 119 d developed no black spots on the shell immediately after thawing (data not shown), they developed melanosis following 3 d of storage at refrigeration temperature. No difference (P > 0.05) was noted in black spot formation among the 4 treatment groups at each frozen storage period. In addition, the longer frozen storage period did not affect black spot formation, although the freshly treated shrimp seemed to have lower readings than those subject to prolonged storage. Yamagata and Low (1995) reported that shrimp stored at −10 °C and −20 °C did not develop any black spots for up to 6 mo. They cited that phenoloxidase, the enzyme responsible for melanosis, was inactive at −10 °C. The enzyme became active again at refrigeration temperature and then contributed to black spot formation on the shrimp after 3 d.

Conclusions

Aqueous chlorine, due to its potent bactericidal activity, has long been used in the food processing plants for control of microorganisms. However, owing to its inherited limitations, such as its causing corrosion of stainless steel and generation of potentially hazardous reaction products, the food industry has been exploring for alternative disinfectant. Electrolyzed oxidizing (EO) water could serve for this purpose because it possesses similar bactericidal activity as the aqueous chlorine. Washing of shrimp with acidic EO water at 40 ppm FAC effectively removed E. coli O157:H7 and Salmonella spp. from the shell surface as the aqueous chlorine or tap water. The treated shrimp by the EO water had significantly lower bacterial loads than the untreated controls at each of the 3 storage days, days 24, 49, and 119, at −20 °C. Both the raw and cooked shrimp that had been treated with acidic EO water showed no difference in sensory attributes as those treated with tap water or aqueous chlorine. Although the EO water treated shrimp after cooking had inferior shrimpy flavor scores, they showed no loss in texture or color following frozen storage for up to 119 d compared with other treatment groups with tap water or aqueous chlorine. The treatment with acidic EO water did not affect the rate of melanosis development on the shrimp.

No bacterial pathogen was found in the treated acidic EO water after washing of the inoculated shrimp. However, residual bacterial loads still existed in the similarly treated aqueous chlorine and tap water. Therefore, the use of acidic EO water to thaw shrimp blocks can reduce not only the bacterial loads on the shrimp but also the opportunity to cross-contaminate another batch of shrimp that needs to be thawed in the same washing solution. EO water can be generated conveniently for on-site uses from a diluted salt solution using a commercial electrolytic cell. This will also reduce the potential hazard of storing large quantities of Clorex in the food processing plants.

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References


References


