

Quaternary ammonium compounds: an alternative disinfection method for fresh produce wash water

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ABSTRACT

Irrigation water can serve as a vehicle for transporting pathogenic microorganisms, and numerous cases of bacterial infections from consumption of irrigated fresh produce have been reported in recent years. Chlorine-based disinfectants applied when produce is packed are widely used to control microorganisms. When applied properly, the chlorine products are effective. However, hazardous disinfection breakdown products can be formed, and chlorine disinfectants have high oxidant activity that can affect produce quality and pose a risk to food handlers. Quaternary Ammonium Compounds (QACs) are a disinfectant alternative for the washing of fruits and vegetables. They can control a great number of microorganisms, have low toxicity when used at recommended doses, and are stable in storage. The purpose of this work was to assess the disinfectant activity of QACs against *Escherichia coli* and *Staphylococcus aureus* under worst-case and average-case turbidity conditions, (2 and 100 nephelometric units); two disinfectant concentrations (100 and 200 mg/L; and two contact times (30 and 120 seconds). Our research showed that QACs were effective against both bacteria. The percentage reduction of *Escherichia coli* was significantly higher in the less turbid solution ($P = 0.027$), while turbidity did not affect the reduction of *Staphylococcus aureus* ($P > 0.05$). *E. coli* was more resistant to QAC treatment than *S. aureus*. Based on the data obtained we can conclude that QACs could be an alternative in washing processes of fruits and vegetables.

Key words | disinfection, *Escherichia coli*, *Staphylococcus aureus*, quaternary ammonium compounds

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INTRODUCTION

In recent years attention has been focused in ways to improve the microbiological quality of fruits and vegetables, particularly on disinfection methods to eliminate human pathogens from fresh produce surface. The number of epidemic outbreaks associated with fresh produce has increased (Tauxe *et al.* 1997; NACMCF 1997; DeWaal *et al.* 2000), attributed principally to sprouts, lettuces, cantaloupes and tomatoes (NACMCF 1997; DeWaal *et al.* 2000). Among the principal pathogens are *Escherichia coli* O157:H7, which in 1996 caused the largest recorded outbreak in which 6,000 cases and 17 deaths occurred due to the consumption of radish sprouts (Beuchat 1996);

Salmonella spp. present in fresh produce, including tomatoes (CDC 1993), cantaloupes (Blostein 1993), and bean sprouts (Beuchat 1998). *Shigella*, *Cyclospora cayetanensis*, Hepatitis A and Norwalk virus have also been detected in fresh produce (Beuchat 1996; NACMCF 1997).

In the past, data about the efficiency of disinfectants was scarce on fresh produce; it was limited only to establishing that the reduction a disinfectant could reach was from 90 to 99% (Beuchat 1998; Brackett 1999). These reductions do not assure the microbial quality of fresh produce. Therefore, there is a need to understand the limiting factors of disinfectant efficiency.

One of the most important activities in the agricultural industry is the search for new technologies that minimize fresh produce contamination. Chlorine is the most used disinfectant in packinghouses since it has the capacity to reduce the presence of pathogenic microorganisms, however there are certain disadvantages, which have led to the search for new disinfection alternatives.

Quaternary ammonium compounds are widely used as disinfectants, antiseptics, pharmaceutical products, and cosmetics and could be an alternative in fruit and vegetables disinfection. All quaternary ammonium compounds (QACs) are cationic compounds that possess a basic structure (NH_4^+). These compounds penetrate into the bacteria cell wall, reacting with the cytoplasmic membrane inducing wall lysis caused by autolytic enzymes (McDonnell & Russell 1999).

QACs are more expensive than chlorine and its derivatives, but they have numerous qualities that make them an attractive alternative for washing fruits and vegetables: they are less affected by organic matter; are not corrosive except at high concentrations; they are stable even in diluted solutions and concentrates, can be stored for a long time without losing their antimicrobial activity (Bougeois *et al.* 1994).

Disinfectant efficiency depends on the type of microorganism (Lisle *et al.* 1998), concentration, contact time, turbidity and temperature (Michael 1991; Bitton 1994; LeChevallier *et al.* 1988; McDonnell & Russell 1999). These factors interact affecting the efficiency of disinfectants. Therefore, to minimize bacteriological risks it is necessary to identify the optimal conditions in which a disinfectant can act at its maximum capacity. A bacterial reduction of $6 \log_{10}$ is needed to establish a disinfectant as effective (Geldreich 1996).

The objectives of this study were: a) To determine the capacity of reduction of quaternary ammonium compounds over a Gram negative (*Escherichia coli*) and a Gram positive (*Staphylococcus aureus*) bacteria; b) To evaluate the effectiveness of quaternary ammonium compounds in low (2 NTU) and high (100 NTU) turbidity conditions; and c) To establish the effective ammonium quaternary compounds concentration to reduce *Escherichia coli* and *Staphylococcus aureus*, assuming cases of low and high turbidity.

MATERIALS AND METHODS

Inoculum preparation and growth conditions

Positive controls of *Escherichia coli* ATCC 15597 and *Staphylococcus aureus* were used in this study. Loops of the stock cultures were transferred to trypticasein soy broth (Difco, Becton Dickinson, Sparks, Md.) and incubated at 37°C for 24 h. Aliquots of the grown cultures were subsequently transferred fresh trypticasein soy broth and incubated again at 37°C for 24 h. Flasks of the resulting *E. coli* and *S. aureus* cultures were centrifuged at 10,000 rpm for 10 min at 4°C. Cell pellets were resuspended in 20 ml of sterile phosphate buffer (0.1 M, pH 7.0) and mixed by a vortex mixer (Fisher Scientific Industries, Inc., Bohemia, N.Y.) for approximately 10 s. The resulting cell suspension was centrifuged again and resuspended as previously described. This concentrated suspension was used to prepare the working suspension in phosphate buffer.

Selective media were prepared under the manufacturer's instructions and used to grow both bacteria; mFC agar (Difco, Detroit MI) and Manitol Salt agar (BD, Bioxon, Mexico) were used to culture *E. coli* and *S. aureus*, respectively.

The spread plate technique was used to culture both bacteria (*Standard Methods* 1998). Samples were incubated at 44.5° C and 37° C during 24 hours for development and enumeration of *E. coli* and *S. aureus*, respectively.

Disinfectant

The quaternary ammonium disinfectant was commercially acquired. QACs are composed by the active ingredients n-alkyl, dimethyl benzyl ammonium chloride, sulfosuccinate dioctyl and urea. The United States Food and Drug Administration (USFDA) authorizes its use for direct contact in equipments and utensils involved in food processing at a concentration of 200 mg/L, however, it cannot be used directly on fresh produce due to the presence of the ingredient n-alkyl, dimethyl benzyl ammonium chloride (21CFR172.165; FDA 2005).

The concentrations used to perform the experiment were 100 and 200 mg/L.

Turbidity

Water concentrations of 2 and 100 NTU were adjusted with Arizona dust and sea salts (General Motors Flint, MI, USA).

Analytic nephelometric equipment was used for turbidity analysis according to *Standard Methods* 1998. The sample was gently mixed and placed into a cell. Turbidity values were read directly expressed in nephelometric units (NTU).

Disinfectant evaluation

A four-liter beaker was used in the disinfectant evaluation; it was filled with one liter of sterile purified water; turbidity was determined with a McVan turbidity meter model 156 and adjusted at 2 and 100 NTU with humic acids (General Motors, Flint, MI, USA), according to the technique established by USEPA (1986). *Escherichia coli* and *Staphylococcus aureus* solutions at known concentrations were added into different beakers; then QACs disinfectant was added at concentrations of 100 and 200 mg/L; a magnetic stirrer (Thermolyne SP46925) was used to stir the sample and simulate the time (30 and 120 seconds) used during the disinfection process in packinghouses.

Design of Experiment

A three factor (concentration, turbidity and contact time) in blocks (bacteria) totally random design was used to analyze the results. The value of response was the percentage of reduction. The effects of the interactions were proved by the Analysis of Variance and means were compared with Tukey test. The statistic software used was MINITAB version 12.

RESULTS AND DISCUSSION

The results are expressed according to Geldreich (1996) who established that a disinfectant is effective when it reduces a 99.9999% of bacterial concentration.

Escherichia coli

The Analysis of Variance showed that the interaction between disinfectant concentration-turbidity and disinfectant concentration-turbidity-time resulted highly significant on the bacterial reduction.

The treatment with the greatest reduction in *E. coli* and the only one that presented significant differences ($P = 0.01$) compared with the rest of the treatments was 100 mg/L – 100

NTU – 30 seconds, obtaining a reduction of 20.78% (Table 1). LeChevallier *et al.* (1988) indicated both inorganic and organic matter enables the bacteria to resist inactivation by disinfectants.

The lowest reduction (54.36%) was obtained by combining the 100 mg/L of QACs and 100 NTU, which had significant differences ($P = 0.001$) when compared with 100 mg/L and 2 NTU (99.9999%), 200 mg/L and 2 NTU (99.9999%), and 200 mg/L and 100 NTU (99.9987%); similar results were obtained by Chaidez *et al.* (2003), who observed that with lower disinfectant concentration and higher turbidity the bacterial reduction was less efficient.

When concentration and contact time interaction was compared, the treatment 100 mg/L of QACs and 30 s (60.38%) was different ($p = 0.018$) from the other treatments with concentrations of 200 mg/L and 30 s (99.9987%), and 200 mg/L and 120 s (99.9999%), showing that the bacterial reduction depended on the disinfectant concentration and not on contact time. Quaternary ammonium compounds were demonstrated to be a disinfectant similar to others widely used in agriculture, reaching a reduction of 99.9999% in the presence of 100 NTU and a contact time of 30 seconds at concentrations of 200 mg/L; comparable to the reduction reported by Foschino *et al.* (1998), who evaluated the bactericidal activity of chlorine dioxide against *E. coli* in water obtaining a 90% reduction, while Rice *et al.* (1999)

Table 1 | Reduction of *Escherichia coli* exposed to ammonium quaternary compounds

QACs concentration ¹	Turbidity ²	Time ³	Percentage of reduction
100	2	30	99.9999 ^c
		120	99.9999 ^c
	100	30	20.7800 ^a
200	2	120	87.9400 ^b
		30	99.9999 ^c
	100	30	99.9975 ^c
200	100	120	99.9999 ^c
		120	99.9999 ^c

Different letters indicates statistical differences.

¹mg/L; Milligrams per liter.

²NTU; Nephelometric Units.

³s; Seconds.

obtained a reduction of 60% in 30 seconds using chlorine against environmental strains of *E. coli* as well as serotype O157:H7.

When comparing the interaction turbidity and time, there were only detected significant differences ($P = 0.018$) between 100 NTU and 30 s against the 2 NTU and 30 s and 2 NTU and 120 s treatments, with a reduction difference of 39.58%, again demonstrating the importance of turbidity in the efficiency of the disinfectant on bacterial reduction.

Staphylococcus aureus

S. aureus did not show statistical differences among the treatments (Table 2). Even more, the analysis of variance revealed that no significant statistical differences existed when contrasting *S. aureus* reduction and *E. coli* in every treatment, except to *E. coli* in the treatment of 100 mg/L, 100 NTU and 30 s, where the reduction obtained was of 20.78%. Similar results were obtained by Shang & Blatchley (2001), who found small differences in chlorine action against Gram positive and Gram negative bacterial suspensions, which were attributed to factors of the cellular wall of the organism. The resistance is due to the outer membrane of Gram negative bacteria which acts like a barrier that limits the entrance to chemical agents.

S. aureus in low and high turbidity and the lowest disinfectant dose did not result in effective treatments based

on the 6 log₁₀ reduction. The study done by Singh et al. (2002), found that in 50% of the *Staphylococcus* spp isolates quaternary ammonium compounds had no effect, due to the presence of resistance genes *qacA/B* and *qacC*; *qac* resistance genes are common and that linkage between resistance to disinfectants and penicillin resistance occurs frequently. In the same way Mayer et al. (2001), have reported *qac* genes in *Staphylococcus* spp, *qacA*, *qacB* and *qacC/smr* which are generally transmitted through plasmids and are widely distributed in the environment. Morton et al. (1995), found the mupirocin resistance plasmid (pG0400) in *S. aureus* isolates, which contain genes encoding resistance to aminoglycosides, trimethoprim and quaternary ammonium compounds. Disinfectants based on quaternary ammonium, e.g. benzalconium chloride, cetylpyridinium chloride, tetramides and detizor, are frequently used in hospitals to disinfect and prevent pathogens spreading. This has suggested that the extensive use of QACs could generate a selective pressure and contribute to the appearance of microorganisms resistant to disinfection (Russell 2000).

Quaternary ammonium compounds at 200 mg/L, resulted in a reduction of 99.9999% of *S. aureus* in any of the turbidity and contact time conditions, but the results were not the same in low and high turbidity conditions in 100 mg/L concentration, where the effectiveness of the disinfectant was reduced.

In general *S. aureus* was reduced by 99.9996% while *E. coli* 88.59%, thus *E. coli* had a higher resistance to the disinfectant action, which coincides with that described by Michael (1991), who found that *S. aureus* susceptibility to cationic agents is greater in comparison to *E. coli*.

Although certain treatments were not statistically different, the reduction obtained by those conditions would be an important achievement for a commercial packinghouse plant.

Table 2 | Reduction of *Staphylococcus aureus* exposed to ammonium quaternary compounds

QACs concentration ¹	Turbidity ²	Time ³	Percentage of reduction
100	2	30	99.9993 ^a
		120	99.9998 ^a
	100	30	99.9988 ^a
		120	99.9998 ^a
200	2	30	99.9999 ^a
		120	99.9999 ^a
	100	30	99.9999 ^a
		120	99.9999 ^a

Different letters indicates statistical differences.

¹mg/L; Milligrams per liter.

²NTU; Nephelometric Units.

³s; Seconds.

CONCLUSIONS

Quaternary ammonium compounds were demonstrated to be an effective disinfectant under most water quality conditions.

The disinfectant effectiveness was reduced by high turbidity (100 NTU) at 100 mg/L; however at 200 mg/L the 6 log₁₀ reduction was reached.

The effective concentration to reduce *Escherichia coli* is 200 mg/L at 100 NTU and 120 s contact time, while at 2

NTU and 30 s CT 100 mg/L of disinfectant is required. For the elimination of *Staphylococcus aureus* 200 mg/L is required at both turbidities conditions with a CT of 30 s.

Although the bactericidal activity observed, in the present study, by the quaternary ammonium compounds was efficient and it could be an option in the washing processes of fruit and vegetables, a greater understanding of the virucidal and protozoacidal action is clearly needed.

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