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RESEARCH ARTICLE

EVALUATION OF THE EFFICIENCY OF DIFFERENT SANITIZERS IN STANDARD STRAINS

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ABSTRACT

Currently the population is more conscientious of the relation between health and feeding, consequence of this is the increase in the consumption of vegetables and fresh fruits. These foods had never been issue of serious concern, therefore they were considered safe. Lately, however, considering the increase of soaring related to contaminated fruits and vegetables, this idea has changed and a lot of researches are being done in order to investigate the microbiological growth, as well as products and methodologies that reduce or eliminate these contaminations. The purpose of this study is to evaluate the effectiveness of three sanitizing agents generally used in domestic and industrial level for the hygienic cleaning of fruits and vegetables, in three different concentrations: sodium hypochlorite at 1%, 2, 5% and 5%, sodium dichoroisocyanurate at 1%, 3% and 5% and vinegar as 6%, 25% and 50%, when applied in standard strains of *Shigella*, *Proteus*, *Salmonella* and *Staphylococcus aureus*. The results evidenced that the sodium dichoroisocyanurate as 3% and 5%, the sodium hypochlorite as 2,5% and 5% and the vinegar as 6%, 25% and 50%, are efficient in the elimination of standard strains. Therefore, it can be evidenced that the process of hygienic cleaning is indispensable to get a safer food, reducing the risk of occurrences of food intoxication and infection caused by pathogenic microrganisms, preserving the health of the commensal.

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INTRODUCTION

The demand for fresh foods, with reduced caloric content, healthy, nutritious and with good quality, has increased considerably (Zambrano, 2006). That can be seen in the market of fresh horticultural products that has grown significantly in the last decade, mainly the segment of products that has been washed, peeled, cut or sliced, packaged uncooked, and stored under refrigeration, known as minimally processed horticultural products (Rodrigues *et al*., 2011; Machado *et al*., 2014).

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Products such as tomatoes, lettuce, cabbage and fruit juices like orange and apple, are currently considered the main horticultural products related to outbreaks of food poisoning in many countries. Especially because these foods have been a source of significant pathogens in public health, such as *Escherichia coli*, *Salmonella* sp., *Shigela* sp., *Listeria* sp., as well as Hepatitis A and parasitic diseases causative agents (Abreu *et al*., 2010). According to Ornellas (2001), due to the fact that these foods grow close to the ground, they are contaminated by soil, insecticides and microrganisms of various kind. Moreover, the use of polluted water for irrigation and inadequate further manipulation aggravate the contamination.

Beuchat (2002) also states that the use of animal manure that has not being composted in the fertilization of these vegetables can also contribute to food contamination even in the country area. To wash the vegetables is the most common practice to provide a safer product, reducing 90% the amount of microrganisms when used flowing water of good quality (Frank; Takeushi, 1999), however it is important to note that the washing is intended only to remove the particles that were in the soil such as soil grains, stones and insects, and it is not intended to eliminate microrganisms (Sapers, 2001), thus being essential the correct sanitization stage (Silva Junior, 2002; Rodrigues *et al*., 2011). The chlorine-based compounds, especially sodium hypochlorite, are the most widely used industrial and household level for the the sanitization of horticultural products and it is recommended by legislation the use of 200-250 ppm, and the contact time required is 15 minutes (Nascimento; Silva; Okazaki, 2003), but there is the risk of formation of undesirable compounds, such as trihalomethanes, which are considered potential carcinogens (Nascimento & Silva, 2010).

Sodium dichloro isocyanurate (NaDCC), a chlorinated organic compound that is sold as powder or effervescent tablet, does not release heavy metals and trihalomethane, when hydrolyzed. It matches a requirement for specific manufacturing process for use in foods. This way, the replacement of inorganic chlorine compounds, by NaDCC, for use in foods, has been consolidated since the 1990's in the United States of America and Europe (Macedo, 2001). Vinegar in Brazil is widely used domestically in the disinfection of fruits and vegetables (Porto & Eiroa, 2006) due to its bactericidal properties (Granada *et al*. 2000), with a minimum concentration of acetic acid of 4% (Nascimento; Silva; Okazaki, 2003). It is recommended the use of acetic acid in aqueous solution at 2% for 15 minutes of contact, and to obtain this concentration is necessary to dilute 500 mL of vinegar per liter of water (Silva Junior, 2002; Nascimento; Silva; Okazaki, 2003).Considering the differences between the different sanitizers, their active compounds and their concentrations, the purpose of this study is to evaluate the efficacy of disinfectants used in sanitizing horticultural products, when applied in different concentrations in standard strains of *Shigella*, *Proteus*, *Salmonella* and *Staphylococcus aureus*.

MATERIALS AND METHODS

In this experiment there were tested three sanitizers commonly used in domestic and industrial level for the sanitization of horticultural products, in three different concentrations: sodium hypochlorite at 1.0%, 2.5% and 5.0%, sodium dichloro isocyanurate at 1.0%, 3.0% and 5.0%, and vinegar at 6.0%, 25.0% and 50.0%. It is valid to note that these concentrations were used because they are the most used by the manufacturer's recommendation, or even used in previous studies done by Nascimento *et al*., 2003; Nascimento; Silva; Okazaki, 2003; Beerli; Vilas Boas; Piccoli, 2004; Lund; Petrini; Rombaldi, 2005; Fontana, 2006; and Nascimento & Silva, 2010. The research began with the determination of the amount of sanitizer required for obtaining each concentration and subsequent preparation of the culture medium PCA (Plate Count Agar). Then, the culture medium was distributed in sterile petri dishes containing each active compounds in their respective concentration. Then, the inocula *Shigella*, *Proteus*, *Salmonella* and *Staphylococcus aureus*were standardized based on the scale 0.5 of McFarland, according to the degree of turbidity of the bacterial suspension. The inocula were then sown by seeding technique on the surface and incubated at 37°C for 24 hours for the reading and counting viable bacteria. Note that plates were prepared containing only the culture medium and inoculum in which were used as control. For the statistical evaluation of the results, Student "t" Test was used, performed in the software Graph Pad Instat, version 3.0 (San Diego, CA, USA). It was considered $p<0.05$ as statistically significant differences.

RESULTS AND DISCUSSION

The results obtained in the evaluation of the efficiency of different sanitizers and different concentrations of standard strains are shown in the Table 1. Using the Student "t" Test to statistically compare the results of the control treatment with the results obtained from *Staphylococcus aureus*, *Salmonella*, *Shigella* and *Proteus*, at concentrations of 1% of sodium dichloro isocyanurate and sodium hypochlorite, it can be concluded that there was no statistically significant difference ($p<0.05$). With these results, it was shown that the treatments in these concentrations showed no efficiency. It is also noteworthy that even comparing the results of these two sanitizers with the control treatment, it is possible to observe that the sodium hypochlorite was more efficient for *Staphylococcus aureus* with reduction of 60.46% compared to 30.23% of sodium dichloro isocyanurate. For *Salmonella* was found that sodium dichloro isocyanurate was more effective with reduction of 51.76% compared to 21.17% of sodium hypochlorite. Regarding *Shigella* and *Proteus*, it was observed that sodium hypochlorite and sodium dichloro isocyanurate had similar reductions with 9.13% and 8.62% for *Shigella*, and 41.15% and 41.92% for *Proteus*, respectively.

It can be seen that the chlorine-based compounds that were tested, both sodium hypochlorite and the sodium dichloro isocyanurate, showed similar performance, demonstrating to be effective for all micro-organisms when tested at concentrations of 2.5% and 5%, and 3% and 5% respectively, in which it was found absence of microbial growth.These results come to corroborate the study by Nascimento *et al*. (2003), in order to compare the efficiency of sodium hypochlorite (200 ppm), sodium dichloro isocyanurate (200 ppm), acetic acid (2% and 4%), peracetic acid (80 ppm) and vinegar (6%, 25% and 50%), after the evaluation of the decimal reductions of microorganisms mesophilic aerobes, yeasts and molds, also total coliforms present in grape samples, was found that sodium hypochlorite and sodium dichloro isocyanurate, both at 200 ppm, showed similar results. Another study by Silva *et al*. (2003) also demonstrated that both the hypochlorite and the sodium dichloro isocyanurate proved to be effective in the destruction of *E.coli* O157:H7 in suspension, in concentrations of 100 ppm and 200 ppm, after 30 seconds of contact.

Fantuzzi; Pushmann; Vanetti (2004), while studying the microbial reduction of minimally processed cabbage immersion, in sanitizing solutions of 200 mg/L of sodium hypochlorite, 0.66% of Sumaveg® and 1% of acetic acid, found a significant reduction of microbial contaminants, wherein the three treatments showed similar results.Ferreira *et al*. (2011) found that the sanitization of *in natura* vegetables, using sodium hypochlorite at 2% for 15 minutes, is effective in reducing at least 97% of the initial microbial load by thermotolerant coliforms and positive *Staphylococcus* coagulase.

Table 1. Average counting of microorganisms submitted to treatment with sodium dichloro isocyanurate, sodium hypochlorite and vinegar at different concentrations

Treatments	Concentrations	S. aureus	Salmonella	Shigella	Proteus
Control	None	0,86 x 10^6	2,55 x10^6	1,97 x 10^6	2,6 x 10^6
	1%	0,60 x 10^6	1,23 x 10^6	1,8 x 10^6	1,51 x 10^6
Sodium dichloro isocyanurate	3%	No growth	No growth	No growth	No growth
	5%	No growth	No growth	No growth	No growth
Sodium hypochlorite	1%	0,34 x 10^6	2,01 x 10^6	1,79 x 10^6	1,53 x 10^6
	2,5%	No growth	No growth	No growth	No growth
	5%	No growth	No growth	No growth	No growth
	6%	No growth	No growth	No growth	No growth
Vinegar	25%	No growth	No growth	No growth	No growth
	50%	No growth	No growth	No growth	No growth

Regarding vinegar, this proved to be efficient in all the concentrations that were tested (6%, 25% and 50%), for all strains in which they were applied, being possible to see that there was no microbial growth.These results confirm the study done by Ivoglo & Rocha (2003) regarding the tests with sodium hypochlorite and vinegar, compared to the micro-organisms present on lettuce. They also found that the most effective disinfectant in the reduction of micro-organisms was vinegar, observing that the sodium hypochlorite can react with other compounds present in the tissues of lettuce, preventing a better efficiency. The study done by Santos *et al*. (2004) with the objective to evaluate the efficacy of various antimicrobial methods in lettuce, goaling to reduce fecal and total coliforms, study that tested washing with water, vinegar and water, and water and sodium hypochlorite, it was found that the more efficient was sodium hypochlorite.

The author also states that other studies have demonstrated that the use of vinegar at 6% was the sanitizer that showed the best results for the reduction of fecal coliforms, total coliforms and *Vibrio cholerae*, when compared to the acetic acid at 0.2% and 0.3%, hypochlorite at 100mg/L and the combination of sodium hypochlorite with vinegar. Leitão *et al*. (1981) analyzed the efficiency of chemical compounds based on chlorine, iodine, chlorine bromine and acetic acid, in the disinfection of lettuce, using various techniques for preparation of samples and different conditions of use of disinfectants. The study found that, in the concentrations and times exposure tested, none of the disinfectants proved able to reduce the bacterial microflora to levels considered safe, being vinegar at 2% more efficient than chlorine and iodine.

Another study done by Nascimento; Silva; Okazaki. (2003), comparing the effectiveness of sodium hypochlorite (200 ppm), sodium dichloro isocyanurate (200 ppm), acetic acid (2% and 4%), peracetic acid (80 ppm) and vinegar (6%, 25% and 50%) by reduction of the population of aerobic mesophiles of fruits and vegetables, showed that all treatments showed similar or superior performance compared to sodium hypochlorite, showing that acetic acid (4%) is the most effective treatment, followed by vinegar (50%), and the less effective is vinegar (6%). Nascimento & Silva (2010) conducted a study in order to compare the efficiency of different sanitizers (red wine vinegar at 50%, 25% and 6% (v/v); acetic acid at 4% and 2% (v/v); peracetic acid at 80 mg.L^{-1}; sodium dichloro isocyanurate 200 at mg.L^{-1}) to sodium hypochlorite at 200 mg.L^{-1} on the microbiota of fresh strawberries. They found that all tested sanitizers can be used as an alternative for the sanitization of fresh fruit, because they presented similar or superior performance to sodium hypochlorite at 200 mg.L^{-1}, that is considered and recommended as the standard sanitizing for horticultural products. The efficiency of chlorine-based and vinegar-based sanitizers used to disinfect samples of lettuces was studied by Moreira *et al*. (2013). They found that the solutions of chlorine and water, and the solution of vinegar and water, in the proportion of 10% for 10 minutes, both were able to reduce the counting of coliforms at 35°C and 45°C. The samples sanitized with vinegar showed result of 4 MPN/g and the samples sanitized with hypochlorite showed result of 2.3 MPN/g, both results were within the limits for fresh *in natura* vegetables. For Zambrano (2006) it is important to note that in the cleaning and sanitizing procedures, a low concentration of sanitizing can allow the appearing of bacterial resistance, while a high concentration can increase costs and can allow contamination of food and environment. Therefore, to Frank and Takeushi (1999), the efficient washing of vegetables using sanitizing added in the water is considered a critical control point, often used in countries like the United States of America. According to Santana *et al*. (2006), it is important to take into account the initial contamination of the product, because this contamination is directly related to the efficiency of the sanitation process. Thus, it should also be noted that greater care must be taken during all the stages, mainly in the rural area production, including transport, storage and handling, in order to obtain a safer product, thus preserving the health of consumers.

Conclusion

Sodium dichloro isocyanurate at 3% and 5%, sodium hypochlorite at 2.5% and 5%, and vinegar at 6%, 25% and 50%, were effective in eliminating the standard strains of *Shigella*, *Proteus*, *Salmonella* and *Staphylococcus aureus*.Considering that most of the vegetables and fruits have high contamination, it is essential that, all the people that work in food and nutrition units, restaurants and especially the housewives, are informed about the potential risks of transmitting pathogens through foods, and the awareness of the importance of the sanitizing step using efficient agents applied in the recommended concentrations and exposure times.

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