Effectiveness of different concentrations of ozonated water in the sanitization of fresh-cut green pepper

Rívia Darla Álvares Amaral*, Mara Lígia Biazotto Bachelli, Mateus TrovóZerbinati, Benedito Carlos Benedetti

(*Universidade Estadual de Campinas – Faculdade de Engenharia Agrícola, Av. Cândido Randon, 501, Cidade Universitária 'Zeferino Vaz' , Barão Geraldo, 13083-875, Campinas, São Paulo, Brazil*)

Abstract: Green pepper is a popular vegetable in Brazil. It is consumed mainly in raw salads or their complements, and therefore should be given special attention as to sanitization. Sodium hypochlorite is currently the sanitizer most used for this purpose, but the formation of by-products is an inconvenience. Thus, the search for techniques to minimize and/or replace this product in the fresh-cut industry has been increased. Ozonated water can be an alternative because it is a potent sanitizer and does not create by-products. The aim of this work was to define the most effective concentration of ozonated water in reducing microbial contamination of fresh-cut green pepper. The raw material was purchased in a local trade of Campinas-SP, selected and minimally processed. The sanitization consisted of immersion in tap water (T_2) and ozonated water at concentrations of 1.6 mg/L (T_3), 1.8 mg/L (T_4) for one min. The processing consisted of washing, manual cutting, taking up the stalk and the internal parts and slicing into strips (± 3 mm in thickness). The control treatment (T_1) was the product minimally processed without washing. The ozone concentration was measured by a commercially available kit (CHEMetrics, Vacu-vials, Ozone K-7402, Calverton, Va., U.S.A.). It was performed physical-chemical (pH and soluble solids) and microbial analysis (mesophilic and psychrotrophic aerobic bacteria, total coliforms, *Escherichia Coli,* yeasts and molds, besides the presence of *Salmonella* spp.). The average initial contamination of samples were 1.3×10^5 CFU/g to mesophilic aerobic bacteria, $>1.1\times10^3$ CFU/g to total coliforms, <1 (est.) to psychrotrophic and yeasts and molds. After the treatment, the results were 1.3×10^4, 9.2 and <1 (est.) CFU/g, respectively. The presence of *E.coli* and Salmonella spp. were not observed. The concentration of 1.6 mg/L was found to be the most effective treatment in reducing the microbial contamination of fresh-cut green pepper.

Keywords: microbial contamination, safe food, postharvest, chemical methods, green-cut peppers

Citation: Amaral, R. D. A., M. L. B. Bachelli, M. T. Zerbinati, and B. C. Benedetti. 2012. Effectiveness of different concentrations of ozonated water in the sanitization of fresh-cut green pepper. Agric Eng Int: CIGR Journal, 14(3): 131−135.

1 Introduction

Minimally processed food (MP) goes through processes such as classification, peeling, cutting and packaging, showing similar quality to fresh product and quick and safe from a microbiological point (Moretti, 2004). These products are increasingly popular among consumers, which see them as healthy and practical. The advantages to consumers are: more convenience in food preparation, reduced waste due to disposal of unwanted parts, greater security in the acquisition of clean and packed products and possibility of purchasing smaller quantities.

One of the most important characteristics is the rapid loss of quality and reduced shelf-life when compared with the role product (Conesa et al., 2007). Fresh-cut vegetables being consumed in the most of cases without cooking may contain pathogenic bacteria that may represent a risk to consumers (Abadias et al., 2008, Han et al., 2000). Green pepper from the field may have a load of microorganisms, which can increase depending

Received date: 2012-05-25 **Accepted date:** 2012-08-28

*** Corresponding author: Rívia Darla Álvares Amaral,** Email: riviamaral@yahoo.com.br.

how the product was handled. Then proper sanitization is essential in reducing microbial contamination (Barros et al., 1994, Gonzalez-Aguiar et al., 2004).

The increase in consumption of MP food and their association with foodborne disease outbreaks raise the safety concerns of these products (Little and Gillespie, 2008). Several studies have been done to detect pathogens in minimally processed fruits and vegetables (Santos et al., 2012, Abadias et al., 2011, Oliveira et al., 2011, Sant´Ana et al., 2011, Abadias et al., 2008). According to SVS (2005) Salmonella was the etiological agent identified in 34.7% of the foodborne outbreaks in the State of São Paulo from 1999 to 2004.

The method most used in Brazil for the sanitization of fruits and vegetables is sodium hypochlorite (Vanetti, 2004), but due to the production of chlorinated organic compounds, like trihalomethanes, which have carcinogenic potential and its impacts on human health and environmental safety has brought upon the search for alternative methods to replace this method sanitization (Garg et al., 1990; Kim et al., 1999; Park and Lee, 1995; Vanetti, 2004).

Ozone is an alternative sanitizer to chlorine and has been very effective on bacteria inactivation, viruses and cysts of *Giardia* and *Criptosporidium*, both protozoa resistant to chlorine (Cavalcante, 2007). It doesn't leave residues in food, because it decomposes quickly in non-toxic molecular oxygen and used in low concentrations in a short time could be enough to obtain similar efficiency or in reducing microbial contamination in operations for sanitization. However, ozone's effectiveness depends on type of product, dose, method of application (ozonated water or gaseous), temperature, pH, relative humidity and presence of organic substances (Prestes, 2007). Ozone treatment (0.18 ppm) can reduce the population of microorganisms and retard physiological metabolism of fresh-cut celery store at 4℃ for nine days of storage (Zhang et al., 2005). Although ozonated water is not a method widely used in minimally processed industry, the substitution of chlorine by the ozone in sanitization of fruits and vegetables would be very important as regards to efficiency in reducing the number of *Enterobacteriaceae*, which include species of hygienic concern. Thereby, it can significantly reduce the consumption of free chlorine and the charge of organic chlorine residues in the waste water (Baur et al., 2004).

In the search for an alternative method to sodium hypochlorite in the sanitization of fresh-cut green pepper, the aim of this work was to define the most effective concentration of ozonated water to reduce microbial contamination of this product.

2 Materials and methods

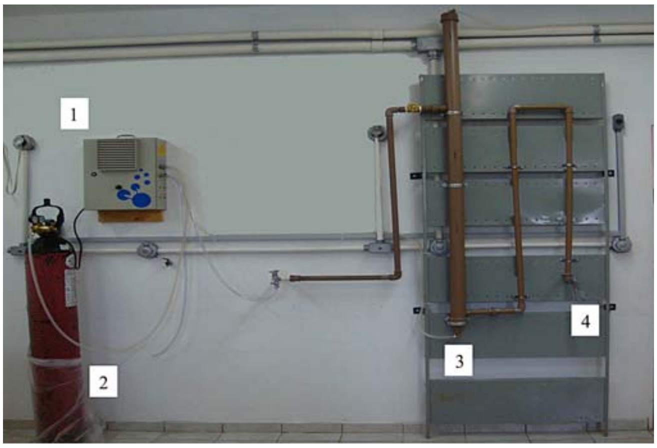
2.1 Raw material and processing

Green pepper was purchased in a local trade market of Campinas, SP, Brazil, selected, considering the lacking of injuries, and transported to the Postharvest Laboratory of the of the Agricultural Engineering Faculty of the University of Campinas. The product was randomly divided into four parcels. The first one was not washed (T_1), the second was washed with tap water (T_2), the third was sanitized with ozonated water at 1.6 mg/L (T_3), and the fourth parcel was sanitized with ozonated water at 1.8 mg/L (T_4). Then, each parcel was minimally processed.

The minimal processing consisted of washing, with the exception of T1, manual cutting, taking up the stalk and the internal parts and slicing into strips (±3 mm in thickness) with a sharp stainless steel knife. After this step, T_1 (unwashed slices), and T_2 (slices washed with tap water) samples were withdrawn. Treatments T_3 and T_4 were washed with ozonated water at concentrations: 1.6 mg/L and 1.8 mg/L, respectively, for one min. After washing, the products were drained for one min and taken for analysis.

All stages of processing were followed by Good Manufacturing Practices, and then the processing area and all the tools contained inside were cleaned. All people involved in the processing have utilized Individual Protected Equipments (IPE's) that consisted of apron, PVC boots, cap, mask and gloves. The temperature of the processing room was maintained at 10±2℃ and the washing water at 7±2℃, excepted to treatment T_2 which used ambient water.

The equipment for the production of ozonated water used in the sanitization process of fruits (Figure 1) recirculated ozonated water at a flow rate of 20 L/min through a stainless steel tank with 50 L capacity and provided a concentration of ozone of 1.0 to 2.0 mg/L. Ozone concentration was measured with an available commercial kit (CHEMetrics, Vacu-vials, Ozone K-7402, Calverton, Va., U.S.A.).



1. Generating ozone cell 2. Oxygen cilinder (99% of pureness) 3. Diffusing pipe 4. Ozonated water exit

Figure 1 Equipment for production of ozonated water,

The experiment was conducted in a completely randomized design with three replicates. Data was submitted to analysis of variance (ANOVA) and the averages compared with Tukey's test (5% probability), using the statistical package *Statistical Analysis System* (SAS Institute Inc., North Carolina, USA, 1989).

2.2 Analysis

2.2.1 Microbiological

Microbial analyses were executed according to methodology described by Silva et al. (2007).

a) Salmonella ssp: traditional technique of detection by classical cultural method of presence/absence, ISO 6579 (2007) method, using a sample of 25 g mixed manually with 225 ml of peptone water, incubated at 37±1℃ (18±2 h)$^{-1}$;

b) Total count of psychrotrophic and mesophilic aerobic bacteria: performed by plating on the surface method, using Plate Count Agar (PCA). The samples were incubated at 35±1℃ (48±2 h)$^{-1}$ for aerobic mesophilic and 7±1℃ (10 d)$^{-1}$ to total count of psychrotrophic aerobic. The reading was done in machine manual colony counter. The results were expressed as colony-forming units per gram of sample (CFU/g);

c) Total count of yeasts and molds: by the standard count method in spread plating, using Dichloran Rose Bengal Chloramphenicol (DRBC) Agar. The samples were incubated at 22-25℃ for five days. The results were expressed as colony-forming units per gram of sample (CFU/g);

d) Total coliforms and *Escherichia coli*: were performed by Most Probable Number method (MPN). The inoculated tubes of Lauryl Sulphate Tryptose (LST) were incubated at 35±0.5℃ (24±2 h)$^{-1}$ and the growth of gas production was observed. The confirmation test was performed with tubes of Billiant Green broth (VB) and *E.Coli* broth (EC).

2.2.2 Physical-chemical

Analyses were carried out according to the methodology described by AOAC (1995).

a) Total soluble solid content: direct reading in digital refractometer, using the homogenate pulp. The results were expressed as ^{o} Brix.

b) pH: direct measurement by potentiometry, which is the immersion of digital pH meter in the homogenized and crushed sample. The results were expressed as pH units.

3 Results and discussion

3.1 Microbial analyses

The effects of different concentrations of ozonated water and the washing with tap water in aerobic mesophilic and psychrotrophic bacteria, yeasts and molds and total coliforms counts are shown in Table 1. The data for *Salmonella* spp. is not shown because its presence was not detected in any sample, confirming that this product does not offer a risk factor for the health of consumers.

Table 1 Microbiological analysis results for the fresh-cut green pepper after the sanitation treatments

Microrganism	Treatment			
	Without washing	Washed with tap water	Ozone at 1.6 mg L^{-1}	Ozone at 1.8 mg L^{-1}
Mesophilic*	1.3×10^{5}	>6.5×10^{6} est	>6.5 ×10^{6} est	1.27×10^{4}
Yeasts and molds*	<10 est.	<10 est.	<10 est.	<10 est.
Psychrotrophic*	<10 est.	<10 est.	<10 est.	<10 est.
Total coliforms**	>1.1×10^{3}	>1.1×10^{3}	9.2×10^{1}	>1. ×10^{3}

Note: Values in CFU/g; ** Values in MPN $^{-1}$ (most probable number); est: estimated value, according to Silva et al. (2007).

The Brazilian Legislation, ANVISA – Resolution RDC-12 (Brasil, 2001), provides a microbiological standard for vegetables and similar - fresh, whole, prepared (peeled or selected or fractionated), sanitized, refrigerated or frozen for consumption, which consists of the absence of *Salmonella* in 25 g of product and maximum of 10^2 CFU/g of coliforms at 45℃. The sanitization process is a very important step in reducing microbial load of whole and fresh-cut products, which was demonstrated in the experiment.

For values of total coliforms, high counts were observed in the treatments without washing in tap water. The sanitization with 1.6 mg/L was efficient, reducing the value of these microrganisms to a value acceptable by the law (Table 1). While the legislation does not establish parameters and neither refers to yeasts and molds (potential foodbornes) and neither the total coliforms (potential foodborne and group of bioindicators of food hygiene), Berbari et al. (2001) argued that populations of total coliforms in level of 10^5 CFU/g and yeasts and molds in level of 10^4 CFU/g corresponded to high contamination of these microorganisms in product. Lower values than the recommendation of Berbari et al. (2001) were found on fresh-cut green pepper in this work.

The concentrations of ozonated water studied were not effective in reducing aerobic mesophilic count. According to Adams et al. (1989), this could be a consequence of neutralization of sanitizers by components leaching from cut produce surfaces.

3.2 Physical-chemical analysis

The results of pH and soluble solids for the fresh-cut green pepper after the treatments are presented in Table 2. The data showed that the fresh-cut green pepper was significantly different in pH among the product without washing, washed with tap water and in different concentrations of the ozonated water; however, the different concentrations were statistically equal between themselves. According to Ragaert et al. (2007), intracellular pH is an important intrinsic factor to fresh-cut vegetables and can vary from 4.9 - 6.9. The pH values found in fresh-cut green pepper in this work were from 6.00 - 6.18. Similar values had been found by Pilon et al. (2006). This range of pH could allow the growth of microorganisms from the moment that the nutrients become available.

Table 2 Results of pH and soluble solids of fresh-cut green pepper without washing, washed with tap water and with 1.6 mg/L and 1.8 mg/L of ozonated water

Analysis	Treatment			
	Unwashed	Washed with tap water	Ozone at 1.6 mg/L	Ozone at 1.8 mg/L
pH	6.18A	6.07B	6.01C	6.00C
Soluble solids (°Brix)	4.50A	4.25B	4.00C	4.25B

Averages followed by distinct capital letters in the same line differ between itself to the level of 5% of probability, for Tukey's test.

For soluble solids content, the product washed with tap water and the sanitized with 1.8 mg/L of ozonated water are statistical equal, however, they are different from the treatment without washing and from the sanitized with ozone at 1.6 mg/L. The highest value was for the treatment without washing (4.50 °Brix) and the minor was the product sanitized with 1.6 mg/L (4.00 °Brix). These values for soluble solids were similar to the values in the range of 3.5 to 5.0 °Brix found by Morgado et al. (2008) when they evaluate different temperatures and coverings in the conservation of "Magali -R" green pepper.

4 Conclusion

The average initial contamination of samples were 1.3×10^5 CFU/g to mesophilic aerobic bacteria, $>1.1\times10^3$ CFU/g to total coliforms, <10(est.) to psychrotrophic and yeasts and molds. After the treatment, the results were 1.3×10^4, 9.2 and < 10 (est.) CFU/g, respectively. The presence of *E.coli* and *Salmonella* spp. was not observed. The concentration of 1.6 mg/L of ozonated water was considered the most effective treatment in reducing the microbial contamination of fresh-cut green pepper.

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