Effectiveness of Different Concentrations of Ozonated Water in the Sanitization of Fresh-Cut Green Pepper

Rívia Darla Álvares Amaral[1], Mara Lígia Biazotto Bachelli[1], Mateus Trovó Zerbinati[1], Benedito Carlos Benedetti[1]

Affiliation:
[1]UNICAMP - Faculdade de Engenharia Agrícola, Av. Cândido Randon, 501, Barão Geraldo, 13083-875, Campinas, São Paulo, Brazil
E-mail: riviamaral@yahoo.com.br

ABSTRACT

Green pepper is a vegetable very consumed in Brazil, mainly in raw salads or their complements, and therefore should be given special attention to sanitization. Sodium hypochlorite is currently the sanitizer most used for this purpose, but the formation of by-products is an inconvenience. Thus, has increased the search for techniques to minimize and/or replace this product in the fresh-cut industry. Ozonated water can be an alternative because it is a potent sanitizer and does not create by-products. The aim of this work was 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 was consisted of immersion in tap water (T_2) and ozonated water at concentrations of 1.6 mg L^{-1} (T_3), 1.8 mg L^{-1} (T_4) for 1 minute. The processing was consisted of washing, manual cut, taking up the stalk and the internal parts and slicing into strips (± 3mm 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 $1.3x10^5$ CFU g^{-1} to mesophilic aerobic bacteria, >$1.1x10^3$ CFU g^{-1} to total coliforms, <1(est.) to psychrotrophic and yeasts and molds. After the treatment, the results were $1.3x10^4$, 9.2 and <1(est.) CFU g^{-1}, respectively. It was not observed presence of E.coli and salmonella spp. The concentration of 1.6 mg L^{-1} was considerate the most effective treatment in reducing the microbial contamination of fresh-cut green pepper.

Keywords: Microbial contamination, washing, ozone, Brazil

Rívia Amaral, Mara Bachelli, Mateus Zerbinati, Benedito Benedetti. "Effectiveness of different concentrations of ozonated water in the sanitization of fresh-cut green pepper". International Commission of Agricultural and Biological Engineers, Section V. Conference "Technology and Management to Increase the Efficiency in Sustainable Agricultural Systems", Rosario, Argentina, 1-4 September 2009.

1. INTRODUCTION

Minimally processed food (MP) go through process of classification, peeling, cutting and packaging, showing similar quality to fresh product and quick and safe from a microbiological point. These products are increasingly popular among consumers, which see them like healthy and practical. 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, becoming a concern (Abadias *et al*., 2008, Han *et al*., 2000). Green pepper can bring from the field a load of microorganisms which depending how the product was handling, can be increase and hold them, the sanitization is essential in reducing of microbial contamination (Barros *et al*., 1994, Gonzalez-Aguiar *et al*., 2004).

The method most used in Brazil for sanitization of fruits and vegetables is sodium hypochlorite (Vanetti, 2004), but due the production of chlorinated organic compounds, like trihalomethanes which have carcinogenic potential and its impacts on human health and environmental safety has been occurred the search of alternative methods in replace to 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 its decompose 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 of microbial contamination in operations for sanitization, but the action of ozone depends on type of product, dose, method of application (ozonated water or gaseous), temperature, pH, relative humidity and presence of organic substances (Prestes, 2007).

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

2. MATERIAL AND METHODS

2.1 Raw material and processing

Green pepper was purchased in a local trade 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 4 parcels. The first one was not washed (T1), the second was washed with tap water (T_2), the third was sanitized with ozonated water at 1.6 mg L^{-1} (T_3), and the fourth parcel was sanitized with ozonated water at 1.8 mg L^{-1} (T_4). Then, each parcel was minimally processed.

The minimal processing consisted of washing, excepted to T1, manual cutting, taking up the stalk and the internal parts and slicing into strips (± 3mm thickness) with a sharp stainless steel knife. After this step, T_1 (not washing slices), and T_2 (slices washed with tap water) samples were withdrawn. In treatments T_3 and T_4 were the washing with ozonated water at concentrations: 1.6 mg L^{-1} and 1.8 mg L^{-1}, respectively, for 1 minute. After washing, the products were drained for 1 minute and taken for analysis.

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

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

Figure 1**.** Equipment for production of ozonated water.

The experiment was conducted in completely randomized design with 3 replications. Data were submitted to analysis of variance (ANOVA) and the averages compared by 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 25g mixed manually with 225ml of peptone water, incubated at 37ºC±1ºC/18±2h;
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 35ºC±1ºC/48±2h for aerobic mesophilic and 7ºC±1ºC/10 days 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^{-1});
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 22-25º C for five days.

The results were expressed as colony-forming units per gram of sample (CFU g^{-1});
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±0,5ºC / 24±2h and the growth with 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 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 pHmeter in the homogenized and crushed sample. The results were expressed as punits;

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. Data for *Salmonella* spp. are not shown because its presence was not detected in any sample, confirming that this product does not offered 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 x 10^5	> 6.5 x 10^6 est	> 6.5 x 10^6 est	1.27 x 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 x 10^3	>1.1 x 10^3	9.2 x 10^1	>1.1 x 10^3

* Values in CFU g^{-1}; ** Values MPN $^{-1}$

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), sanitizated, refrigerated or frozen for consumption, which consists of the absence of *Salmonella* in 25 g of product and maximum of 10^2 CFU g^{-1} of coliforms at 45 °C. The sanitization process is a very important step on reducing microbial load of whole and fresh-cut products, which was demonstrated in the experiment performed.
For values of total coliforms, high counts were observed in the treatments without washing and washing in tap water. The sanitization with 1.6 mg L^{-1} 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 foodbornes and group of bioindicators of food hygiene), Berbari *et al*. (2001) argue that populations of total coliforms on level of 10^5 CFU g^{-1} and yeasts and molds on level of 10^4 CFU g^{-1} correspond to high contamination of these microorganisms in product. Lower values, than the recommendation of Berbari *et al*. (2001), were found to fresh-cut green pepper in this work.

The concentrations of ozonated water studied were not effective in reducing of 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 significant different in pH of 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 to 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^{-1} and 1.8 mg L^{-1} of ozonated water.

Analysis	Treatment			
	Without washing	Washed with tap water	Ozone at 1.6 mg L^{-1}	Ozone at 1.8 mg L^{-1}
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^{-1} 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^{-1}. The highest value was for the treatment without washing (4.50 °Brix) and the minor was the product sanitized with 1.6 mg L^{-1} (4.00 °Brix). Morgado *et al*. (2008) to evaluate different temperatures and coverings in the conservation of "Magali -R" green pepper found levels of soluble solids in the range 3.5 to 5.0 °Brix.

4. CONCLUSION

The average initial contamination of samples were 1.3×10^5 CFU g^{-1} to mesophilic aerobic bacteria, >1.1×10^3 CFU g^{-1} 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^{-1}, respectively. It was not observed presence of E.coli and Salmonella spp. The concentration of 1.6 mg L^{-1} of ozonated water was considerate the most effective treatment in reducing the microbial contamination of fresh-cut green pepper.

5. REFERENCES

ABADIAS, M., USALL, J., ANGUERA, M., SOLSONA, C., VIÑAS, I. 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *International Journal of Food Microbiology,* 123, 121–129.

ADAMS, M.R., HARTLEY, A.D., COX, L.J. 1989. Factors affecting the efficacy of washing procedures used in the production of prepared salads. *Food Microbiol*., 6, 69–77.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (AOAC). 1995. *Official methods of analysis*. 17 ed., Arlington. 1141 p.
BARROS, J.C.S.M., GOES, A., MINAM, K. 1994. Condições de conservação pós-colheita de frutos de pimentão *(Capsicum annum* L.). *Sci. Agric*., 51(2), 363-368.
BERBARI, S.A.G., PASCHOALINO, J.E., SILVEIRA, N.F.A. 2001. Efeito do cloro na água de lavagem para desinfecção de alface minimamente processada. *Ciência e Tecnologia de Alimentos*, Campinas, 21(02), 197-201.
BRASIL. 2001. Ministério da Saúde. Agência Nacional de Vigilância Sanitária – ANVISA. Resolução RDC-12/01, de 2 de janeiro de 2001. Diário Oficial [da] República Federativa do Brasil. Brasília, DF, 10 jan. 2001. Seção 1, p.45.
CAVALCANTE, D.A. 2007. *Avaliação do tratamento com água ozonizada para higienização de alface* (*Lactuca sativa*). Campinas. 102f. Dissertação (Mestrado em Tecnologia de Alimentos) – Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas.
CONESA, A., VERLINDEN, B.E., ARTÉS-HERNÁNDEZ, F., NICOLAI, B., ARTÉS, F. 2007. Respiration rates of fresh-cut bell peppers under supertamospheric and low oxygen with or without high carbon dioxide. *Postharvest Biology and Technology*, 45, 81–88.
GARG, N.; CHUREY, J.J.; SPLITTSTOESSER, D.F. 1990. Effect of processing conditions on the microflora of fresh vegetables. *Journal of Food Protection*, 53(08), 701-703.
GONZÁLEZ-AGUILAR, G.A., AYALA-ZAVALA, J.F., RUIZ-CRUZ, S., ACEDO-FÉLIX, E.,DÍAZ-CINCO, M.E. 2004. Effect of temperature and modified atmosphere packaging on overall quality of fresh-cut bell peppers. *Lebensm.-Wiss. u.-Technol*., 37, 817-826.
HAN, Y., SHERMAN, D.M., LINTON, R.H., NIELSEN, S.S., NELSON, P.E. 2000. Efects of Escherichia coli O157: H7 to green pepper surfaces. *Food Microbiology,* 17, 521-533.
KIM, J.G.; YOUSEF, A.E.; DAVE, S. 1999. Aplication of ozone for enhancing the microbilogical safety and quality of foods: a review. *Journal of Food Protection*, Des Moines, 62(09), 1071-1087.
MORGADO, C.M.A.; DURIGAN, J.F.; SANCHES, J.; GALATI, V.C.; OGASSAVARA, F.O. 2008. Conservação pós-colheita de frutos de pimentão sob diferentes condições de armazenamento e filmes. *Horticultura Brasileira*, 26, 170-174.
PARK, W.P.; LEE, D.S.1995. Effect of chlorine treatment on cut watercress and onion. *Journal of Food Quality*, 18, 415-424.
PILON, L., OETTERER, M., GALLO, C.R., SPOTO, M.H.F. 2006. Minimally processed carrot and green pepper. *Ciênc. Tecnol. Aliment*., Campinas, 26(01), 150-158.
PRESTES, E.B. 2007. *Avaliação da eficiência do ozônio com sanitizante em hortaliças folhosas minimamente processadas***.** Campinas. 135f. Dissertação (Doutorado em Tecnologia de Alimentos) - Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas.
RAGAERT, P., DEVLIEGHERE, F., DEBEVERE, J., 2007. Role of microbiological and physiological spoilage mechanisms during storage of minimally processed vegetables. *Postharvest Biology and Technology.* 44, 185–194.
SILVA, N., JUNQUEIRA, V.C.A. SILVEIRA, N.F.A. 2007. *Manual de Métodos de Análise Microbiológica de Alimentos*. Livraria Varela, 3ªed.
VANETTI, M.C.D. 2004. Segurança microbiológica em produtos minimamente processados. *In: III Encontro Nacional sobre Processamento Mínimo de Frutas e Hortaliças*. Resumos. Viçosa: Universidade Federal de Viçosa. 242p., 30-32.