

**INFLUENCE OF DISINFECTANT TECHNOLOGIES ON RED BELL  
PEPPERS SAFETY**

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## ABSTRACT

The development of innovative technologies, promoting fruits and vegetables safety to reduce the risk of related food-borne diseases, is an actual concern. Several sanitizing agents for washing fruits and vegetables may be used to reduce the risk of microbial contamination. Traditional washing technologies utilize chlorine and hydrogen peroxide as sanitizing agents for fresh produce. More recently, ozone is being used as an innovative food processing technology that guarantees product safety, also increasing shelf life of fruits and vegetables. Its main advantage, when compared to traditional disinfectant technologies, relies on its potent antimicrobial action and non-toxic products decomposition.

The objective of this work was to study the effectiveness of three sanitizing agents (sodium hypochlorite - used in a commercial available solution AMUKINA, hydrogen peroxide and ozone in aqueous solutions), on the reduction of *Listeria innocua* inoculated on red bell peppers.

Results showed that ozone treatment allowed bacteria reductions identical to the ones attained with the traditional disinfectant solutions. On average, aqueous ozone allowed a decimal reduction of 2 cycles in *Listeria innocua* counts.

**Keywords:** fruits and vegetables, safety, innovative technologies, ozone, hydrogen peroxide, sodium hypochlorite.

## INTRODUCTION

The occurrence of outbreaks of food-borne illness represents serious public health problems. Among the foods with higher incidence of contamination are raw fruits and vegetables. Prevention of preharvest contamination is important to minimise the risk of disease caused by consuming hazardous raw produce, since postharvest treatments with sanitizers may not be efficient in pathogens elimination, especially when the contamination is high. Among pathogens (such as *Salmonella*, *E.coli*, *Pseudomonas*,

*Campylobacter*), *Listeria monocytogenes* is one of the most widespread microorganism of food-borne infections. The bacteria is widely distributed in the environment and has been isolated from a variety of foods, such as vegetables, milk, cheese, fish, meat and poultry products. As it tolerates high concentrations of salt, extreme temperature and pH conditions, it can be isolated from ready-to-eat food products.

Several sanitizing agents for washing fruits and vegetables may be used to reduce the risk of microbial contamination. Traditional washing technologies utilize chlorine as sanitizing agent for fresh produce. Commonly, it may be added to wash-water as sodium or calcium hypochlorite. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is another well studied oxidant agent, directly toxic to pathogens. More recently, ozone is being used as an innovative food processing technology that guarantees product safety, also increasing shelf life of fruits and vegetables (Rice et al. 1982). Its main advantage, when compared to traditional disinfectant technologies, relies on its potent antimicrobial action and non-toxic products decomposition (Kim et al. 2003; Dufresne et al. 2004).

The effectiveness of disinfectant technologies depends on the microbial sensitivity to the sanitizer agent used, and consequently variable results are commonly reported by researchers. Investigation on specific pathogens/produce combinations is lacking.

Generally, chlorine concentration used to wash fresh produces rounds 50 to 200 p.p.m.. Ukuku & Sapers (2001) refer a 3-log reduction of *Salmonella* in cantaloupes (a sort of melon), when washed with a chlorine solution. For the product, Ukuku et al. (2001) reported 2 and 3-4 log-reductions of the indigenous surface microflora and *E. coli*, respectively.

Ukuku et al. (2005) studied the effect of hydrogen peroxide also in cantaloupe and honeydew melons inoculated with *Listeria monocytogenes* and *E. coli*. Sapers et al (1999) reported a 2.5 log reduction in *E.coli* load of apples, when washed with hydrogen peroxide solutions.

Ozonation is often referred as efficient for food quality and safety (Khadre et al. 2001; Guzel-Seydim et al. 2004; Manousaridis et al. 2005). However, published results of ozone in aqueous solution are not so promising. As an example, Ketteringham et al. (2006) mentioned a 0.72 log reduction of Aerobic Plate Counts in green peppers. Such reductions were considered too small to be commercially viable.

The objective of this work was to study the effectiveness of three sanitizing agents (sodium hypochlorite, hydrogen peroxide and ozone in aqueous solution) on the

reduction of *Listeria innocua* inoculated on red bell peppers. *Listeria innocua* was chosen as the target microorganism, because apart from being non-pathogenic, it is physiologically very close to *L. monocytogenes*, and both can be isolated in the same food products.

## **MATERIAL AND METHODS**

### **Sample preparation**

Red bell peppers (*Capsicum annuum*, L.) were acquired in a local market. They were pre-washed in current tap water for a few seconds, and dried with absorbant paper. Peduncles and seeds were removed and samples were cut in small portions of approximately 20 g.

Each sample was artificially inoculated at the surface, with 250 µL of the second *Listeria* subculture (see section *Listeria innocua* culture). Contact time was 15 minutes.

### ***Listeria innocua* culture**

*Listeria innocua* NCTC 10528 was acquired commercially from Leatherhead Food Research Association (Leatherhead, UK). The first subculture was made in TSB, Tryptic Soy Broth (Lab M, Lancashire, UK), containing 0.6% yeast extract (Lab M, Lancashire, UK) – TSBYE - at 30°C during 24h. The cultures were maintained at 7°C on Tryptic Soy Agar, TSA (Lab M, Lancashire, UK), supplemented with 0.6% yeast extract - TSAYE.

The second subculture of *Listeria innocua* was prepared like the first one at 30°C for ± 24h to yield stationary phase cultures. This cell growth phase was chosen, because the stress resistance of *Listeria innocua* cells is higher in that phase in comparison with the lag or exponential phases.

## **Disinfectant treatments**

Artificially contaminated red bell peppers were washed in water (used as control), in ozonated water ( $O_3$  concentration of approximately 2 p.p.m.), and solutions of sodium hypochlorite (11.5 g/L; used in a commercial available solution - AMUKINA) and hydrogen peroxide ( $H_2O_2$  solutions at 1% and 5% w/w). Contact time of all treatments was 2 minutes and, at least, 4 replicates were carried out. The ratio between mass of samples and volume of disinfectant solution was 20 g/L.

Pilot equipment with continuous ozone production was used in assays. An ozone generator (OZ5, SPO3, Sociedade Portuguesa de Ozono, Portugal) interconnected to a container (volume around 30 L; the ratio between mass of samples and volume of ozonated water was 20 g/30 L) filled with tap water, formed a closed circuit rig apparatus. Ozone was continuously incorporated in water (at  $\sim 15^\circ C$ ) and its content was indirectly measured by potential difference (SZ 265, B&C Electronics).

## **Microbiological analysis**

After each treatment, samples were aseptically cut in small pieces and homogenised in a stomacher using 80 mL of Buffered Peptone Water, BPW (Lab M, Lancashire, UK), for 5 minutes. Decimal dilutions were carried out in BPW, and *Listeria* enumeration was assessed, in duplicate, using Palcam agar containing selective supplement (Merck, Darmstadt, Germany). Samples were incubated at  $30^\circ C$  during 3 days, for posterior counts.

## **Data analysis**

The treatment effects were assessed by calculation of log-reduction of microbial content, in relation to untreated samples.

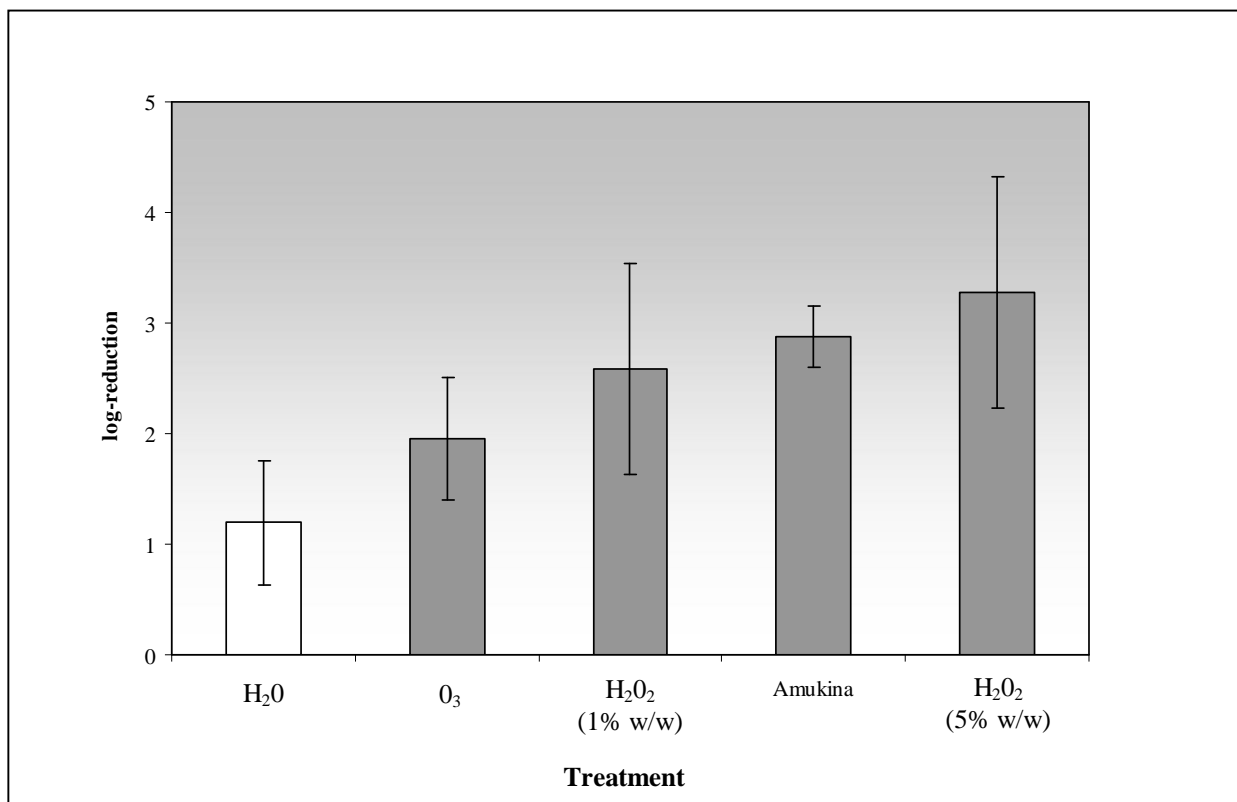
An analysis of variance was performed (one-way ANOVA), using SPSS<sup>®</sup> 14.0 for Windows<sup>®</sup> (2006 SPSS Inc., Chicago, USA).

## RESULTS AND DISCUSSION

The artificial inoculation procedure allowed red bell pepper contaminations around  $10^7$  CFU/g. The objective of such procedure was to guarantee a high level of samples contamination and, therefore, potentially to reduce microbial content by action of the disinfectant treatment applied.

Results of *Listeria innocua* log-reductions in red bell peppers, in relation to untreated ones, are presented in figure 1. No significant differences were detected between the washing disinfectants used (ANOVA; significance level of 5%). The mean of bacteria log-reductions fell within 2-3 log-cycles.

When compared to water-washings, ozonated water and solutions of sodium hypochlorite and hydrogen peroxide allowed higher microbial reductions. In average, water-washings reduced  $1.2 \pm 0.6$  log-cycles ( $\pm$  standard deviation) of *Listeria innocua* in red bell peppers.



**Figure 1.** Effect of disinfectant treatments on microbiological reduction of *Listeria innocua* in red bell peppers (the bars indicate standard deviation of experimental values).

The presence of *Listeria innocua* was inspected in all disinfectant solutions and washing waters, before and after treatments. Bacteria were only detected in water, after washing of the samples.

## CONCLUSIONS

The use of ozone in aqueous solutions allowed identical reductions in *Listeria innocua* of red bell peppers, when compared to traditional disinfectant solutions of hydrogen peroxide and sodium hypochlorite. In average, all treatments allowed a reduction of 2-3 log-cycles, which was higher than the reduction observed with water-washings.

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