Modelling microbial load reduction in foods due to ozone impact

Elisabete M.C. Alexandre, Teresa R.S. Brandão, Cristina L.M. Silva *

CBQF / Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal

Abstract

Ozone is a strong sanitizer that can be applied as a convenient washing-treatment to foods. The main objective was to study the ozone impact on *Listeria innocua* in red bell peppers, total mesophiles in strawberries and total coliforms in watercress. Modelling microbial load reduction throughout treatment time and due to ozone effect were also targets. The microbiological reductions observed for ozonated samples were higher than the ones obtained for water dipping. However, total coliforms/watercress were less sensitive to both deionized-water and ozonated-water washings. A Weibull-based model was adequate in describing microbiological reductions and may contribute to design more effective sanitizing processes.

Keywords: Ozone; microbial contamination; fruits and vegetables; modelling

1. Introduction

Fruits and vegetables contain a great diversity of microbial flora and are frequently involved in food-borne outbreaks. Mesophilic microorganisms, coliforms, yeasts and molds are populations commonly found in those products, which are responsible for quality degradation and safety compromise. Since fruits and vegetables are often consumed uncooked or unwashed, the microbiological contamination should be reduced to minimize risks.

Blanching is one of the most effective processes for inactivation of microorganisms and enzymes responsible for quality decay in fruits and vegetables, thus resulting in products with extended shelf life. Although it has the main disadvantage of degrading sensorial and nutritional attributes and, from an industrial point of view, this process may be extremely energy consuming [1]. Alternatively, challenging non-thermal technologies such as UV-C radiation, ultrasounds and ozone treatments may be promising,

* Corresponding author. Tel.: +351-22-5580058; fax: +351-22-5090351.
E-mail address: clsilva@esb.ucp.pt.

since quality can be better retained while safety, from a microbiological point of view, can also be attained.

Ozone, due to its powerful oxidizing effect, is one of the most potent disinfectant agents. In 1997 it was recognized by U.S. Food and Drug Administration as a GRAS substance (i.e. Generally Recognized as Safe) for use as a disinfectant or sanitizer in foods and food processing [2]. Ozone is effective against some gram positive bacteria (e.g. *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus* and *Enterococcus feacalis*), gram negative bacteria (e.g. *Pseudomonas aeruginosa* and *Yersinia enterocolitica*), some yeasts (e.g. *Candida albicans* and *Zygosaccharomyces bacilli*) and some spores like *Aspergillus niger* [3-5]. When dissolved in water, ozone has been applied as a convenient washing treatment of fruits and vegetables, promoting shelf life extension of food products [6, 7]. Besides several studies assess the ozone impact at microbial loads [8, 9], scarce information is available on modelling the kinetic behavior of food contaminants. Such models may contribute to determine the extent to which the process should be applied in order to improve safe standards and process design.

The main goal of this work was to study the impact of ozone in aqueous solution on the following combinations of microorganisms and foods: *Listeria innocua* in red bell peppers, total mesophiles in strawberries and total coliforms in watercress. Modelling of microbial load reduction throughout treatment time and due to ozone effect were also objectives.

2. Materials & Methods

2.1 Listeria innocua cultures

Listeria innocua NCTC 10528, obtained from Leatherhead Food Research Association (Leatherhead, UK), was subcultured (30°C, 24h) in Tryptic Soy Broth (Lab M, Lancashire, UK), containing 0.6 % yeast extract (Lab M, Lancashire, UK). The cultures were maintained at 7°C on Tryptic Soy Agar (Lab M, Lancashire, UK) supplemented with 0.6% yeast extract.

Stationary phase culture of the bacterium was used in this study.

2.2 Fruits and vegetables samples

Strawberries (*Fragaria ananassa* D.), watercress (*Naturtium officinale* R.Br.) and red bell peppers (*Capsicum annuum* L.) were purchased in a local market.

Strawberries and watercress were cut in portions of 20 g each. Samples were not pre-washed. Native total mesophiles were evaluated on strawberry and total coliforms in watercress.

Red bell peppers were pre-washed in deionized water and were cut in portions of 20 g. Each sample was artificially inoculated at the internal surface, dropping 250 µL of *L. innocua*. The contact time was 15 min.

2.3 Washing treatments

Ozone treatments were performed in a pilot plant, using a corona discharge ozone generator (OZ5, SPO3 - Sociedade Portuguesa de Ozono, Lda., Porto, Portugal). The ozone flow rate was 5 g/h. The gas was continuously incorporated in deionised water (approximately 30 L at 15ºC) and the concentration in aqueous solution was 0.3 ppm. The ozone concentration was controlled by potential difference (Redox probe; SZ 275, B & C Electronics, Carnate, Italy) and was confirmed using an ozone determination kit (25180-50 Ozone AccuVac colour disc kit, HACH Lange GmbH, Düsseldorf, Germany).

Samples were immersed in ozonated water (80g / 30L) and were removed after different contact times. Simple deionized water-washings (without ozone) were performed under identical conditions.

2.4 Microbial enumeration

Microbial populations were assessed in fresh (untreated) and treated samples. 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 done in BPW.

Enumeration of *L. innocua*, total mesophiles and total coliforms was carried out according to ISO 11290-1, ISO 4833:1991, ISO 7218:1996 and ISO 4832:1991 [10-13].

2.5 Modelling procedures

A Weibull-based model was assumed for microbial load reduction throughout time [14, 15]:

$$log\left(\frac{N}{N_0}\right) = 1 - e^{\left(\frac{t}{\alpha}\right)^{\beta}} \qquad (1)$$

where N is the microbial load (the index 0 indicates initial values, i.e. untreated samples) at time t; α is a scale parameter (the reciprocal of the rate constant) and β is a shape index.

The experimental data were fitted to the previous equation, using SPSS 17.0 software (2008 SPSS Inc., Chicago, USA).

3. Results & Discussion

The impact of deionized-water and ozonated-water washings on *L. innocua* in red bell peppers (initial load averaged 1.9 x 10^7 cfu/g), total mesophiles in strawberries (initial load averaged 5.6 x 10^7 cfu/g) and total coliforms in watercress (initial load averaged 3.9 x 10^8 cfu/g) can be observed in Figure 1. Total coliforms were selected for watercress, since these microorganisms are contaminants of this vegetable. Total mesophiles were chosen for strawberries, as representative microorganisms mixture of a fruit. *Listeria innocua* was selected as an indicator microorganism. This bacterium is often used as a surrogate of the pathogenic *L. monocytogenes*.

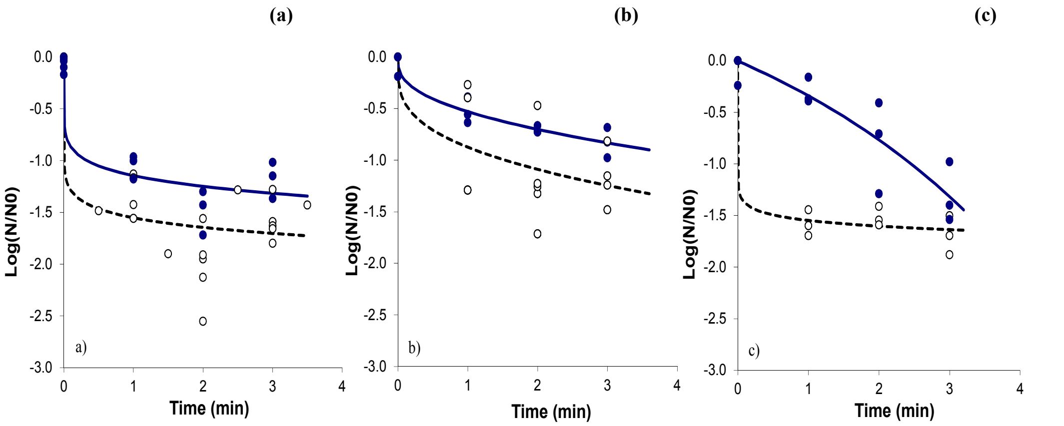

Fig 1. Effect of water and ozonated-water washings on log-reductions of: (a) *L. innocua*/peppers, (b) total coliforms/watercress and (c) total mesophiles/strawberries. Lines are model fits (------ water; - - ozone)

The Weibull-based model was satisfactorily used in data fitting and parameter estimates are in Table 1. Quality of regressions was assessed by residual analysis (randomness and normality of residuals were verified) and on the coefficient of determination, R^2. The values of R^2 were considerable high for almost all cases, varying from 0.83 to 0.96. However, for total coliforms/watercress treated with ozonated-water, a great dispersion of experimental data was observed and the coefficient of determination was 0.71.

It is interesting to observe that parameters β estimated for *L. innocua*/red bell peppers washed in water and in ozonated-water were of the same magnitude, revealing identical curve shapes; this was also observed for total coliforms/watercress. In those cases, the curves concavities were upward. For mesophiles/strawberries washed in water, the sanitising curve was concave down and β parameter was considerably higher (β=0.96). In some cases, parameter estimates lack precision, since meaningless large confidence intervals at 95% were obtained. This can be overcome by using a statistically-supported experimental design [16].

Table 1. Parameter estimates and corresponding confidence intervals at 95% obtained from the best fitting of data using the Weibull model

Microorganism / Food	Treatment	Regression results α (min)	β	R^2
Listeria innocua / Red bell pepper	Water washing	22.3±111.0*	0.09±0.18*	0.92
	Ozonated-water washing	3.3±5.5*	0.05±0.12*	0.84
Total coliforms / Watercress	Water washing	14.3±17.3*	0.32±0.20	0.94
	Ozonated-water washing	7.5±15.0*	0.23±0.34*	0.71
Total mesophiles / Strawberry	Water washing	3.6±0.9	0.96±0.62	0.83
	Ozonated-water washing	7.6±39.6*	0.03±0.12*	0.96

*Meaningless large confidence interval

For all combinations of microorganism/food studied, the impact of ozonated-water washings at microbial loads was higher than the one observed when a simple water-washing was carried out, for all treatment times considered. This means that higher reductions were attained when ozone was used as sanitising agent.

However, a substantial portion of the microbial populations were reduced by water washing alone, and the presence of ozone generally added an additional reduction of approximately 0.4 log-cycles for all times considered, and for the combinations *L. innocua*/red bell peppers and total coliforms/watercress.

The difference between water and ozonated-water washings was particularly evident for total mesophiles/strawberries, and for short contact times (Figure 1c). As an example, for 1 minute of dipping, water-washings allowed 0.3 log-cycles reduction and the presence of ozone added an additional reduction of 1.2 log-cycles.

Total coliforms in watercress were less sensitive to both types of washings.

The differences between the responses observed for total mesophiles and total coliforms due to ozone treatments can be explained by the diversity of native flora enumerated in each case, and consequent different sensitivity to the ozone oxidizing effect. The nature and composition of food surface, the degree of attachment to or association of microorganisms with food and biofilms formation are other possible justifications.

4. Conclusions

Ozonated-water washings are more effective in reducing microbial loads of the fruits and vegetables studied, when compared to simple water dipping. Total coliforms in watercress are less sensitive to both deionized-water and ozonated-water washings.

A Weibull-based model was adequate in describing the reduction of microbial loads and may contribute to design more effective sanitizing processes.

Acknowledgments

The authors acknowledge the financial support through *Programa Operacional Agricultura e Desenvolvimento Rural – Projecto AGRO nº822 (Novas Tecnologias de Processamento de Hortofrutículas Congelados – EMERCON*). The authors Alexandre E.M.C. and Brandão T.R.S. would like to thank *Fundação para a Ciência e a Tecnologia* (grants SFRH/BD/16042/2004 and SFRH/BPD/11580/2002, respectively).

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