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Modeling growth of *Escherichia coli* O157:H7 in fresh-cut lettuce treated with neutral electrolyzed water and under modified atmosphere packaging

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ABSTRACT

The purpose of this study was to evaluate and model the growth of *Escherichia coli* O157:H7 in fresh-cut lettuce submitted to a neutral electrolyzed water (NEW) treatment, packaged in passive modified atmosphere and subsequently stored at different temperatures (4, 8, 13, 16 °C) for a maximum of 27 days. Results indicated that *E. coli* O157:H7 was able to grow at 8, 13, and 16 °C, and declined at 4 °C. However at 8 °C, the lag time lasted 19 days, above the typical shelf-life time for this type of products. A secondary model predicting growth rate as a function of temperature was developed based on a square-root function. A comparison with literature data indicated that the growth predicted by the model for *E. coli* O157:H7 was again lower than those observed with other disinfection treatments or packaging conditions (chlorinated water, untreated product, NEW, etc.). The specific models here developed might be applied to predict growth in products treated with NEW and to improve existing quantitative risk assessments.

1. Introduction

Over the last decades, there has been a great increase in the consumption of ready-to-eat (RTE) and minimally processed fruits and vegetables, because these food products combine healthiness with easy and fast preparation.

Although the prevalence of bacterial pathogens in fruits and vegetables is low as compared with other food products (Doyle and Erickson, 2008; Francis et al., 1999; Garg et al., 1990; Gómez-López et al., 2008), RTE fruits and vegetables can represent a potential health risk due to the fact that no heat treatment is included in their production chain, and the only step aimed to reduce the microbial load are washing and sanitizing treatments (Artés et al., 2009; Beuchat, 2002). Recent foodborne disease outbreaks linked to the consumption of RTE vegetables include cases of *Escherichia coli* O157:H7 (Denmark in 2010; Netherlands in 2007; and Sweden in 2005), and also *E. coli* O104 in Germany in 2011 (Wu et al., 2011). Apart from the public health consequences, food-borne outbreaks can also lead to important losses in the food industry, due to changes in consumer confidence, and effects in trade flows consequence of political decisions (de Vocht et al., 2011).

In the production of RTE vegetables, sodium hypochlorite is the most widely used sanitizer (Akbas and Ölmez, 2007; Behrsing et al., 2000). However, concerns related to its efficacy and the formation of potentially hazardous by-products have provoked an intense research effort aimed to find alternative treatments more effective and that could generate lower quantities or less harmful disinfection by-products. One of the proposed alternatives is to use electrolyzed water (EW) as sanitizer (Guentzel et al., 2008). EW is obtained by conducting an electric current through water containing dissolved NaCl. During this process, electrolysis takes place producing oxidizing agents with strong antibacterial activity such as hypochlorous acid (Artés et al., 2009). This technique gets similar results using lower concentrations of free chlorine, and as a consequence, less disinfection by-products would be generated (Alegria et al., 2009; Al-Haq et al., 2005; Chang et al., 2000). There are different types of EW depending on if the solution is formed at the anode or cathode. If EW is generated at the anode, it is called acidic electrolyzed water (AEW), acid oxidizing water, or electrolyzed oxidizing water. In turn, if it is produced at the cathode, it is known as basic electrolyzed water (BEW), alkaline electrolyzed water, or electrolyzed reducing water. Neutral electrolyzed water (NEW), with a pH around 7, is produced by mixing the anodic solution with an alkaline solution or by using an only single-cell chamber, without separation between both electrodes (Hricova et al., 2008). NEW has demonstrated better features than AEW since it is equally effective in reducing microbial load with lower

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free chlorine levels, while reducing corrosion of surfaces and minimizing human health and environmental issues derived from the volatility of Cl_2, which is high in AEW due to the low pH (Akbas & Ölmez, 2007; Alegria et al., 2009; Al-Haq et al., 2005) (Rahman et al., 2010; Hricova et al., 2008). To this respect it has been found that the major efficacy of NEW as compared with AEW is due to a higher content of the radical species $^{\bullet}OH$ in the same available chlorine concentration level (Xiong et al., 2010).

In spite of the need of predicting growth of *E. coli* O157:H7, with accuracy, in leafy green vegetables, to date few predictive models have been developed on the food matrix itself (i.e. vegetables). Koseki and Isobe (2005) developed a model for *E. coli* O157:H7 growth on non-packaged iceberg lettuce as a function of temperature (5–25 °C) and more recently, a growth model for the pathogen in fresh-cut lettuce submitted to washing with chlorinated water and packaged in modified atmosphere was developed (Posada-Izquierdo et al., 2013). In addition, two growth models based on scientific literature data concerning *E. coli* O157:H7 in fresh-cut leafy vegetables have been developed (Danyluk and Schaffner, 2011; McKellar and Delaquis, 2011). These models incorporate growth data from different leafy green vegetables and under different processing conditions thus producing a more general model. Anyhow, it would be expected that different processing conditions lead to different growth patterns during storage. Therefore, specific models assessing the growth of pathogens after different decontamination treatments would be needed in order to perform more accurate quantitative risk assessments on RTE vegetables. The aim of this study was to evaluate and model the effect of a treatment with neutral electrolyzed water on the subsequent growth/survival of *E. coli* O157:H7 in modified atmosphere packaged lettuce at different storage temperatures.

2. Materials and methods

2.1. Bacteria and preparation of cell suspensions

In the present study, a five-strain cocktail of *E. coli* O157:H7 (CECT 4267, 4076, 4782, 4783, and 5947) provided by the Spanish Type Culture Collection (CECT, Valencia, Spain) was used. Previously, each strain was made resistant to 50 μg of Nalidixic acid (Nal^R strains) per mL (Merck, Darmstadt, Germany), by transferring and incubating (37 °C; 20 h) the microorganism successively in Brain Heart Infusion (BHI, Oxoid, Basingstoke, UK) tubes with increasing concentrations of the antibiotic.

To prepare the five-strain cocktail for experiments, Nal^R strains were separately subcultured twice at 37 °C for 20 h in 5 mL of BHI with Nalidixic acid (Nal). In order to remove BHI, cultures were centrifuged at 4100 rpm for 10 min, and the supernatant was removed and replaced by 0.1% $NaHCO_3$ solution. This procedure was repeated three times. Afterward, suspensions of each strain were mixed in equal volumes to have the same concentration of each of the strains (approximately 10^9 cfu/mL). Levels of *E. coli* O157:H7 in the inoculum were confirmed by enumeration in Chromocult coliform agar (Merck, Barcelona, Spain) supplemented with 50 μg Nal per mL agar (Nal^+) incubated at 37 °C for 24 h.

2.2. Processing, cutting and inoculation of fresh iceberg lettuce

Fresh iceberg lettuce (*Lactuca sativa* L.) acquired from a local market in Murcia (Spain) was processed and cut in a pilot plant refrigerated at 4 °C simulating the industrial conditions applied in the production of fresh-cut lettuce. Outer and damaged leaves were manually removed, and the rest was cut in pieces of 3 × 3 cm approximately. The inoculation process was carried by dividing the total amount of lettuce (14 kg) in 4 lots of 3.5 kg each. The three first lots were inoculated by immersion for 1 min in separate propylene tanks containing 10 L of cold (4 °C) tap water with a concentration of 5×10^6 cfu/mL of the cocktail of Nal^R *E. coli* O157:H7. The fourth lot was not submitted to inoculation since it was used as control in the experiment in order to discard the existence of natural contamination of *E. coli* O157:H7. Subsequently, lettuce was centrifuged to eliminate the excess of water by means of a manually-operated closed spinner centrifuge (Paragourmet 90005), and kept at 4 °C for 1 h before the disinfection treatment in order to facilitate attachment of the pathogen on the lettuce tissue. As a consequence of this process, an inoculum of 5 log cfu/g was obtained on lettuce just before the disinfection treatment.

2.3. Decontamination treatments

Neutral electrolyzed water (NEW) was generated by a pilot scale prototype borrowed from the company Adamant Technologies (La Chaux-de-Fonds, Switzerland). A constant flow of cold water (4 °C) with a concentration of 1 g/L NaCl (Merck, Barcelona, Spain) was pumped through an electrolytic cell with Boron-doped diamond (BDD) coated electrodes. Amperage was kept at a level of 6.4 A, and the current density applied was 24 mA/cm^2. The obtained solution was diluted with tap water in order to prepare a volume of 40 L of a solution with a concentration of 50 mg/L free chlorine, a pH of 6.5 (adjusted with ~3 g citric acid to improve disinfection efficacy of chlorine), an oxidation reduction potential (ORP) of >450 mV (López-Gálvez et al., 2012) and a temperature of 4 °C. Lots of 3.5 kg of inoculated lettuce were washed for 30 s in 40 L of the NEW solution, and rinsed with 40 L of cold tap water (4 °C) also for 30 s. Finally, the excess of water was removed with the same manual centrifuge mentioned above, by applying 50 rpm for 1 min, approximately.

An additional experiment was carried out to assess the effect of the washing/rinsing step without addition of NEW (i.e. using just tap water) on the reduction of *E. coli* O157:H7 on fresh-cut lettuce. For that, a lot of 3.5 kg lettuce was submitted to the same experimental set-up as that used for the above-mentioned NEW experiments, excepting that no NEW was added to the washing water.

Temperature, pH, and ORP in the wash-water were measured by means of a multimeter pH & Redox 26 (Crison, Barcelona, Spain), whereas free and total chlorine were determined based on the N,N-diethyl-p-phenylendiamine (DPD) method (APHA, 1998) using the Spectroquant NOVA 60 photometer (Merck, Darmstadt, Germany).

2.4. Packaging and storage

Samples of 50 g of treated lettuce were packed in passive modified atmosphere in 12 × 12 cm bags simulating commercial proportions of 23 × 30 cm bags containing 250 g. Bags were made of oriented polypropylene (OPP) with a thickness of 35 μm and a permeability to O_2 of 1100 $mL/m^2 \cdot day \cdot atm$ at 23 °C. An amount of 56 bags were stored at each temperature (8, 13 and 16 °C) in controlled temperature chambers (Tecnidex, Valencia, Spain). Additionally, a smaller amount of bags was stored at 4 °C. During the storage, temperature in the chambers was controlled every 30 min. The evolution of gas composition inside the bags was measured with a gas analyzer (Systech Instruments GASPACE 2, 5979, London, UK) each sampling time before microbiological analysis. As passive modified atmosphere was used, the initial composition of gasses in bags corresponded to the air composition, that is to say, 20.9% O_2, 78% N_2, and 0.03% CO_2. However, this composition could not be exactly observed as consequence of the respiration rate of vegetable tissues, which slightly modified the composition inside bags before analysis at time 0.

2.5. Microbiological analysis

Sampling was performed at different time intervals, which were determined based on preliminary experiments about growth of the pathogen on lettuce (data not shown). Sampling points were more distant in time at low temperatures, and were closer at high temperatures. At each sampling point, 6–8 bags were taken from each temperature to be