Food Microbiology 27 (2010) 375–380

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Effects of packaging type and storage temperature on the growth of foodborne pathogens on shredded 'Romaine' lettuce

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ARTICLE INFO

Article history:
Received 26 May 2009
Received in revised form
13 November 2009
Accepted 17 November 2009
Available online 26 November 2009

Keywords:
Salmonella
Escherichia coli O157:H7
Listeria monocytogenes
Fresh-cut vegetables
Modified atmosphere packaging

ABSTRACT

Fresh produce can be a vehicle for the transmission of pathogens capable of causing human illnesses and some of them can grow on fresh-cut vegetables. The survival and growth of *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* inoculated onto shredded lettuce was determined under modified atmosphere packaging conditions, at various storage temperatures. We also monitored changes in pH and gas atmospheres within the packages and the growth of psychrotrophic and mesophilic microorganisms. After pathogen inoculation, shredded lettuce was packaged in films of different permeability and stored at 5 and 25 °C. After 10 days at 5 °C populations of *E. coli* O157:H7 and *Salmonella* decreased approximately 1.00 log unit while *L. monocytogenes* increased about 1.00 log unit, in all package films. Moreover, the pathogens level increased between 2.44 and 4.19 log units after 3 days at 25 °C. Psychrotrophic and mesophilic bacteria had similar growth at both temperatures with higher populations in air than in the other atmospheres. The composition of the storage atmosphere within the packaging of lettuce had no significant effect on the survival and growth of the pathogens used in this study at refrigeration temperatures. The results obtained can be considered as a warning indicator, which reinforces the necessity for corrective measures to avoid contamination of vegetables.

1. Introduction

Production and consumption of minimally processed (ready-to-eat) lettuce has increased dramatically in many countries in recent years. The convenience of cut, prewashed, packaged lettuce benefits consumers and has created a demand for high quality products (Brecht, 1995). In Spain, the consumption per capita in 2006 of fresh-cut fruits and vegetables was still low (1–1.5 Kg) compared with the rest of Europe and USA (Anonymous, 2007). With the increase of consumption, the incidence of foodborne outbreaks caused by contaminated fresh fruits and vegetables has increased in recent years (Mukherjee et al., 2006). Fresh produce can be a vehicle for the transmission of bacterial, parasitic and viral pathogens capable of causing human illnesses and a number of reports refer to raw vegetables harbouring potential foodborne pathogens (Beuchat, 1996; Nguyen-The and Carlin, 1994). *Listeria monocytogenes* (Schlech et al., 1983), *Salmonella* (Doyle, 1990), and *Escherichia coli* (Nguyen-The and Carlin, 1994) have been isolated from raw vegetables, which can become contaminated while growing or during harvesting, postharvest handling, or distribution. Food safety criteria for fresh-cut fruits and vegetables is regulated by the Commission Regulation EC N 2073/2005 (OJEU L338/1-26, 22 December 2005) which has been modified by EC N 1441/2007 (OJEU L322/12-29, 7 December 2007). Those criteria are absence of *Salmonella* in products placed on the market during their shelf life and absence of *L. monocytogenes* in 25 g before the food has left the immediate control of the food business operator who has produced it and <100 cfu g^{-1} in products placed on the market during their shelf life.

The preparation of fresh-cut products causes damage to plant tissues, reducing shelf-life of the more perishable products, compared to the intact fruits and vegetables (Guerzoni et al., 1996; Watada et al., 1996). This problem is primarily due to a higher respiration rate and the significant damage resulting from cutting (Pirovani et al., 1997). Modified atmosphere packaging (MAP) is the alteration of the gaseous environment resulting from produce respiration (passive MAP) or from the addition and removal of gases from food packages (active MAP) to manipulate the levels of oxygen (O_2) and carbon dioxide (CO_2). MAP has been successfully used to maintain the quality of fresh-cut fruit and vegetables. It can affect the type and growth rates of microorganisms present on the produce (Day, 1992) e.g. it may enhance the growth of *L. monocytogenes* (Francis and O'Beirne, 1998). Fresh-cut produce can

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doi:10.1016/j.fm.2009.11.014

modify the atmosphere in their packages as a result of O_2 consumption and CO_2 production (Pirovani et al., 1998). In general, gas compositions inside an MA package depend primarily on temperature, product fill weight, respiration rate, O_2 and CO_2 transmission rates of the package film, and the total respiring surface area (Bolin and Husxoll, 1991; Heimdal et al., 1995; López-Gálvez et al., 1997; Willox, 1995; Zagory and Kader, 1988). Different films have been used to create modified atmospheres, with polyvinylchloride (PVC) being one of the most commercially used (Robertson, 1993). The marketing temperature recommended for the MAP of vegetables is 3 °C, but these products are often stored at 10 °C, an abusive temperature (Day, 1992). The aim of this study was to evaluate the potential of *L. monocytogenes*, *Salmonella* and *E. coli* O157:H7 to grow in shredded lettuce packaged in two types of modified atmosphere packaging (MAP) and in air, as well as the growth of mesophilic and psychrotrophic microorganisms at 5 °C and 25 °C.

2. Material and methods

2.1. Preparation of inocula

The non-pathogenic strain of *E. coli* O157:H7 (NCTC 12900), and the strain BAA-709 (ATCC) of *Salmonella choleraesuis* subsp. choleraesuis (Smith) Weldin serotype Michigan were used. Both strains were adapted to grow on Tryptone Soy Agar (TSA, Oxoid) supplemented with 100 μg mL^{-1} of Streptomycin (St, Sigma). Cultures grown on TSA-St at 37 °C for 20–24 h were inoculated into a flask with 50 mL of Tryptone Soy Broth (Oxoid) supplemented with Streptomycin (TSB-St) at 150 rpm for 20–24 h at 37 °C.

The strain of *L. monocytogenes* serotype 1/2a was isolated from bagged fresh-cut iceberg lettuce in our laboratory (Abadias et al., 2008) and was identified by the "Servicio de Bacteriologia, Centro Nacional Microbiologia, Instituto de Salud Carlos III" (Majadahonda, Madrid, Spain). *L. monocytogenes* was grown in TYSEB (TSB amended with 6.0 g L^{-1} of yeast extract) for 20–24 h at 37 °C.

The cultures were harvested by centrifugation at 9820 × g for 10 min at 10 °C and resuspended in sterile saline peptone (SP, 8.5 g L^{-1} NaCl and 1 g L^{-1} peptone). Concentrations were determined with a spectrophotometer set at $\lambda = 420$ nm according to the standard curves.

2.2. Sample preparation

"Romaine" lettuce (*Lactuca sativa* var. Longifolia) was obtained from a local supermarket in Lleida (Spain). The outer leaves and core of the lettuce were removed and discarded. The remaining leaves were cut into pieces with a sharp knife. All the shredded leaves were washed in cold tap water for approximately 1 min. The excess surface water remaining on the leaves was removed by a manual kitchen centrifuge.

2.3. Inoculation of samples

The shredded lettuce was divided in four batches of approximately 1000 g. Three of them were inoculated by dipping into a 3 L tank containing each of the pathogens studied at 10^5 cfu mL^{-1} for 2 min. The other batch was left uninoculated (control). In addition, a dip in 10^3 cfu mL^{-1} of *L. monocytogenes* was made. These concentrations of cells were estimated to be necessary to allow accurate enumeration by direct plating. The batch that served as control was dipped into 3 L deionized water for 2 min. Inoculated lettuce was let to dry for 30 min in a laminar flow biosafety cabinet.

The actual concentration of each pathogen in the dip tank was determined by plating dip suspension on the selective media. *E. coli* O157:H7 and *Salmonella* Michigan were plated on TSA-St followed by incubation at 37 °C for 20–24 h *L. monocytogenes* was enumerated on Palcam (Biokar Diagnostics) agar media after incubation at 37 °C for 48 h. All analysis was carried out in triplicate packages for each microorganism and for each temperature/gas condition and the experiment was repeated twice.

2.4. Packaging of samples

Three different atmosphere conditions were studied: two different passive modified atmospheres and air condition. For this, samples were packaged in two different polypropylene plastic films (Amcor Flexibles, Ledbury, Herefordshire UK). Film I (35 μ in thickness) had O_2 and CO_2 permeability of 3500 cm^3 m^{-2} day^{-1} atm^{-1} at 23 °C and water steam permeability of 0.9 g m^{-2} day^{-1} at 25 °C and 75% relative humidity. Film II (35 μ in thickness) had O_2 and CO_2 permeability of 1100 cm^3 m^{-2} day^{-1} atm^{-1} at 23 °C and water steam permeability of 0.9 g m^{-2} day^{-1} at 25 °C and 75% relative humidity. To create air conditions, film was manually perforated with five holes of 400 μm each (Film III).

Uninoculated and inoculated samples were weighted (15 ± 1 g) in different film bags (12 cm × 20 cm). Bags were sealed and one-half were stored at 5 °C for 10 days and the other half were stored at 25 °C for 3 days for subsequent evaluation of microbial growth.

2.5. Microbiological analyses

Populations of *E. coli* O157:H7, *Salmonella*, *L. monocytogenes*, mesophilic and psychrotrophic bacteria were determined in three sample bags at each time. The samples from each pathogen and the control treatments were examined on the day of inoculation (d0) and after 1, 2 and 3 days for samples stored at 25 °C, and 2, 6, 8 and 10 days for samples stored at 5 °C. For the analysis, 10 g of lettuce from each bag were mixed with 90 mL of SP in sterile Stomacher bag and homogenized in a Stomacher 400 (Seward, London, UK) set at 230 rpm for 2 min. Further ten-fold dilutions were made with the same diluent. For the inoculated samples, colonies of *E. coli* O157:H7 and *Salmonella* were enumerated by plating onto TSA-St and the plates were incubated at 37 °C for 20–24 h. *L. monocytogenes* colonies were enumerated on Palcam medium incubated at 37 °C for 48 h. In the uninoculated samples, mesophilic and psychrotrophic microorganisms were determined by enumerating colonies on plates with Nutrient Agar (NA, Biokar Diagnostics) incubated at 30 ± 1 °C for 3 days and at 6.5 ± 1 °C for 10 days, respectively.

2.6. Gas analysis and measurement of pH

Throughout the experiment, CO_2 and O_2 concentrations in the bags were analyzed using a handheld gas analyzer (CheckPoint O_2/CO_2, PBI Dansensor, Denmark). The pH of all samples (10 g lettuce + 90 ml SP) was measured after homogenization using a pH-meter (Model GLP22, Crison).

3. Results

3.1. Survival of E. coli O157:H7 in modified atmosphere at various temperatures

The initial populations of *E. coli* on shredded lettuce after inoculation and drying were 4.48, 4.53 and 3.87 log cfu g^{-1} for Film I, Film II and Film III, respectively (Fig. 1).

Populations of *E. coli* O157:H7 on lettuce slightly decreased during the 10-day storage period at 5 °C, whereas they increased after 3 days at 25 °C. Significant increases in populations (2.12, 2.92 and 2.62 log cycles, for Film I, Film II and Film III, respectively) were