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Effects of process severity on survival and growth of *Escherichia coli* and *Listeria innocua* on minimally processed vegetables

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Abstract

The effects of different slicing methods on subsequent growth and survival of *Escherichia coli*, *Listeria innocua*, and background microflora, during storage (8 °C) on modified atmosphere packaged vegetables (sliced carrots, sliced iceberg and butterhead lettuce) were evaluated. *E. coli* and *L. innocua* were used as models for *E. coli* O157:H7 and *Listeria monocytogenes*. Gas atmospheres within packages of minimally processed vegetables (MPV) were monitored to identify any effects of slicing treatments on oxygen and carbon dioxide levels. In general, the slicing method had no significant effect on initial inoculation levels. *L. innocua* grew better and *E. coli* survived better on vegetables sliced with blades that caused the most damage to cut surfaces. Slicing manually with a blunt knife or with machine blades gave consistently higher *E. coli* and *L. innocua* counts during storage than slicing manually with a razor blade. The effects of hand tearing were similar to slicing with a razor blade. The slicing method also affected the growth of the total background microflora; razor sliced vegetables tended to have lower counts than other treatments. Results also indicated that product respiration was affected by slicing method.

Keywords: *E. coli*; *Listeria*; Minimally processed vegetables

1. Introduction

Minimally processed fruit and vegetable products are widely available and generally considered safe to eat by consumers. However, the majority of these products require no further treatment and are eaten raw, posing a potential safety problem. These products may be contaminated with foodborne pathogens such as *Escherichia coli* O157:H7 and *Listeria monocytogenes*. Outbreaks of *E. coli* O157:H7 infection have been linked to fresh vegetables such as alfalfa sprouts, radish sprouts, and different lettuce varieties; infection with enterotoxigenic *E. coli* has been linked to carrots, while *L. monocytogenes* has been associated with infection from the consumption of raw cabbage used in the manufacture of coleslaw (Beuchat, 1996; De Roever, 1998). Both of these pathogens have been isolated from a variety of fruits and vegetables including leafy vegetables, cabbage, celery, cucumber, radish, tomato and beansprouts (Beuchat, 1996) although modified atmosphere packaged (MAP) products have not been implicated in any reported outbreaks.

Shredding and slicing or cutting processes are important sources of contamination of minimally processed produce. Shredding and slicing were found to increase counts of mesophilic bacteria from 10^3–10^4 to 10^5–10^6 CFU g^{-1} for a range of vegetables (Garg, Churey, & Splittstoesser, 1990). Accelerated growth and spoilage occurs due to increased nutrient availability and larger surface areas for microbial growth (Brackett, 1992). These processes bring microorganisms present on the surface into contact with the wounded produce tissue (Garg et al., 1990; Nguyen-the & Carlin, 1994). During

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processing, the mechanical damage caused to cells limits the shelf life of minimally processed fruits and vegetables (King & Bolin, 1989) and provides more entrance points for food borne pathogens.

Bacteria occurring on leaf surfaces are localized in particular sites, most commonly at stomata, bases of trichomes, epidermal cell wall junctions and in grooves along the veins (Beattie & Lindow, 1999). Electron microscopy identified that both upper and lower surfaces of unwashed lettuce leaves were largely coated with bacteria and other debris. Washing in tap water removed bacteria and debris from exposed surfaces, although substantial numbers remained in hollows at the junction of epidermal cells and in folds in the epidermis (Adams, Hartley, & Cox, 1989). Seo and Frank (1999) identified *E. coli* O157:H7 cells attached to the surface, trichomes, stomata and cut edges of lettuce leaves using confocal scanning laser microscopy. Bacteria located in these sites may be protected from disinfection treatments. Several studies (Han, Linton, Nielsen, & Nelson, 2000a; Han, Sherman, Linton, Nielsen, & Nelson, 2000b; Liao & Cooke, 2001; Liao & Sapers, 2000; Seo & Frank, 1999; Takeuchi & Frank, 2000; Takeuchi & Frank, 2001) have identified that the efficacy of various disinfectant treatments depend on the nature of bacterial attachment. It is important therefore, to understand the interactions between food borne pathogens and plant structure so that procedures for more effective disinfection can be put in place.

Slicing vegetables changes the surface morphology of the product and, in doing so, changes the surfaces available for bacterial colonisation. Slicing fruit and vegetable products with different blades or manual tearing of leafy produce affects the nature of the surface provided to pathogens for attachment and possible penetration. Work carried out by Barry-Ryan and O'Beirne (1998) on the effects of slicing methods on the quality and shelf-life of fresh cut carrot slices identified that the use of a sharp blade minimized physical damage. The effects of slicing were influenced by sharpness of blade; machine slicing was more severe than razor slicing. Bolin and Huxsoll (1991) reported that cut and torn surfaces of lettuce examined by scanning electron microscopy looked similar, but that the sliced surface possibly had more cellular debris.

The present study was undertaken to identify the effects of different slicing methods on the survival and growth of *E. coli* and *L. innocua* during storage on packages of sliced carrots, butterhead and iceberg lettuce. Vegetables were inoculated with *E. coli* or *L. innocua* after slicing to mimic mid/post processing contamination. *L. innocua* was used in lieu of *L. monocytogenes* and has been used in previous studies as a model organism for *L. monocytogenes* (Francis & O'Beirne, 1997; Houtsma, Kusters, de Wit, Rombouts, & Zwietering, 1994; Omary, Testin, F, Barefoot, & Rusting, 1993). Francis and O'Beirne (1997) and Francis and O'Beirne (1998) have shown that *L. innocua* NCTC 11288 is a valid model for *L. monocytogenes* behaviour on minimally processed lettuce. Non-pathogenic *E. coli* was used as a possible model for *E. coli* O157:H7. There have been no reports of significant differences in survival and growth characteristics between *E. coli* O157:H7 and non-pathogenic *E. coli* (McClure & Hall, 2000; Yokoigawa, Takikawa, & Kawai, 1999) in minimally processed vegetables (MPV). However, more evidence is needed to show that the strain used is an appropriate model i.e. research needs to be conducted to determine whether there are suitable characteristic similarities in growth and survival patterns.

2. Materials and methods

2.1. Plant materials

Locally grown Irish carrots (cultivar Nairobi) were used for the production of modified atmosphere packaged carrot slices. They were washed prior to storage by the producer. Carrots were used in experiments from October to February. Irish butterhead lettuce (November–December) and iceberg lettuce (April–August) imported from Spain were used for production of modified atmosphere packaged sliced lettuce. Samples were from different batches but from the same supplier.

2.2. Preparation of vegetables

Carrots, iceberg and butterhead lettuce were purchased from a local supplier and stored overnight at 8 °C. The next day, a sequence of processing steps was used for the preparation of MPV (Fig. 1).

Carrots (5 kg) were topped and tailed using a sharp knife and then hand peeled. The end slices were discarded. Carrots were sliced into 6 mm thick discs, either manually or by machine. Backed stainless steel razor blades were used for hand slicing. A Sammic CA 300 vegetable processing machine (Barcelona, Spain) equipped with either a sharp or a blunt cutting disk, was used for mechanical slicing. Previously unused sharp blades were used for this experiment. The blunt blades were rendered blunt after use on a coleslaw production line for 1 year. Carrot slices (25 g) were transferred aseptically into bags (15×6 cm), made using oriented polypropylene film (35 μm).

Outer and damaged leaves as well as the core were removed from heads of iceberg and butterhead lettuce. Leaves of iceberg and butterhead lettuce were hand torn or sliced using a backed stainless steel razor blade into approximately 15 mm strips. Leaves of butterhead lettuce were also hand sliced using a blunt knife. Iceberg and butterhead lettuce strips (25 g) were transferred