The impact of a cold chain break on the survival of *Salmonella enterica* and *Listeria monocytogenes* on minimally processed 'Conference' pears during their shelf life

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ABSTRACT

BACKGROUND: In recent years, improved detection methods and increased fresh-cut processing of produce have led to an increased number of outbreaks associated with fresh fruits and vegetables. During fruit and vegetable processing, natural protective barriers are removed and tissues are cut, causing nutrient rich exudates and providing attachment sites for microbes. Consequently, fresh-cut produce is more susceptible to microbial proliferation than whole produce.

RESULTS: The aim of this study was to examine the impact of storage temperature on the growth and survival of *Listeria monocytogenes* and *Salmonella enterica* on a fresh-cut 'Conference' pear over an eight day storage period. Pears were cut, dipped in antioxidant solution, artificially inoculated with *L. monocytogenes* and *S. enterica*, packed under modified atmospheric conditions simulating commercial applications and stored in properly refrigerated conditions (constant storage at 4 °C for 8 days) or in temperature abuse conditions (3 days at 4 °C plus 5 days at 8 °C). After 8 days of storage, both conditions resulted in a significant decrease of *S. enterica* populations on pear wedges. In contrast, when samples were stored at 4 °C for 8 days, *L. monocytogenes* populations increased 1.6 logarithmic units, whereas under the temperature abuse conditions, *L. monocytogenes* populations increased 2.2 logarithmic units.

CONCLUSION: *Listeria monocytogenes* was able to grow on fresh-cut pears processed under the conditions described here, despite low pH, refrigeration and use of modified atmosphere.

Keywords: foodborne pathogen; ready-to-eat; fruit; cold.

INTRODUCTION

Processed products attractive to consumers could be a way to increase consumption of fruits and vegetables, with a positive impact on consumer health if processing does not alter the nutritional benefits of the raw products.[1] Minimally processed pears could satisfy consumer demand because pears have low protein and lipid contents and are rich in sugars, including fructose, sorbitol, and sucrose, and are low in glucose. Pears also contain micronutrients, including vitamins (vitamin C, vitamin E, and niacin) and minerals (potassium, phosphorous, calcium, and magnesium).[2] However, fruit processing promotes faster deterioration due to tissue damage, which leads to increased physiological activity and major physicochemical changes, including enzymatic browning, softening, and tissue degradation.[3] Several investigators have developed technologies to minimize these processing effects.[4-15] Use of antioxidant solutions and edible coatings together with a modified atmosphere package (MAP) can reduce surface browning and water loss.[16] In addition to improving processing techniques to maintain quality, precautions should be taken to ensure product safety. The potential for microbiological contamination of fruits and vegetables is high because of the wide variety of conditions to which produce is exposed during growth, harvest, processing, and distribution. It is well established that fresh produce may contain high contamination levels after harvest. During processing, spoilage and pathogenic microorganisms can also contaminate the product surface, and the nutrients inside the fruit contribute to their growth.[3] Thus, disinfection is one of the most important processing steps affecting the quality, safety, and shelf life of the end product.[17] Safety requirements related to fresh-cut produce include good agricultural practices (GAP) and good processing practices (GMF) that result in the absence of pathogens, mycotoxins, pesticide residues, and any other chemical or physical contamination that might risk consumer health.[16] In the Europe Union, food safety criteria for the presence of microorganisms in food is regulated by EC N° 2073/2005 and subsequent amendments. The food safety criteria for *L. monocytogenes* are of particular interest on ready-to-eat (RTE) foods. In RTE foods that may support the growth of

L. monocytogenes, the limit is 100 CFU g^{-1} during the shelf life and the absence of *L. monocytogenes* in 25 g of the food just before it has left the immediate control of the food business operator who produced it. In RTE foods unable to support the growth of *L. monocytogenes*, the limit is also 100 CFU g^{-1} during the shelf life.[18]

In recent years, improved detection methods and increased fresh-cut processing of produce have led to an increased number of outbreaks associated with fresh fruits and vegetables.[19] The storage temperature is an important factor affecting the growth of microorganisms. Thus, effective cold chain management is critical for maintaining the quality and shelf life of the product. The aim of this study was to examine the impact of storage temperature on the growth and survival of *Listeria monocytogenes* and *Salmonella enterica* on a fresh-cut 'Conference' pear during its shelf life.

MATERIALS AND METHODS

Fruit

'Conference' pears (*Pyrus communis* cv. Conference) were obtained from local packing houses in Lleida (Catalonia, Spain). The guide for minimally processing 'Conference' pears used in this paper was based on previous work.[20] Prior to processing, the pears were disinfected by immersion in a 0.1 g L^{-1} sodium hypochlorite (NaClO) solution (pH 6.5) for 2 min, rinsed in running tap water and allowed to dry at room temperature. Each pear was peeled and cut into 10 wedges using a handheld apple corer and slicer. In some fruit pieces, a 6 mm diameter well was made at the centre of each wedge for the inoculum. All the 'Conference' pear wedges were treated with an optimum antioxidant solution (20 g L^{-1} calcium ascorbate plus 10 g L^{-1} calcium chloride solution) by immersion for 2 min in the solution (1:2 w/v), which was maintained on a rotating platform at 150 rpm. The treated pear wedges were allowed to dry in a laminar flow biosafety cabinet for a short time. Physicochemical characteristics (soluble solids content and titratable acidity) of the pear wedges were evaluated in triplicate after the antioxidant treatment. The pear wedges were squeezed, and the soluble solids content (SCC) was determined using a handheld refractometer (ATAGO CO., LTD, Japan) at 20 °C. Results were reported as °Brix.

To measure titratable acidity (TA), triplicate samples of 10 mL of fruit extract were diluted with 10 mL of distilled water, and 2 drops of phenolphthalein solution, 0.1 mL L^{-1} (Panreac, Barcelona, Spain) were added. The mixture was titrated with sodium hydroxide solution (NaOH, 0.1 N) until the pH indicator changed colour. The results were calculated as g of malic acid per litre of juice.

Foodborne pathogens

The bacterial strains used in this work included the serovars of *Salmonella enterica* subsp. *enterica:* Agona (ATCC BAA-707), Michigan (ATCC BAA-709), Montevideo (ATCC BAA-710) and Gaminara (ATCC BAA-711) in addition to the *L. monocytogenes* serovar 1a (CECT 4031), serovar 3a (CECT 933), serovar 4d (CECT 940), serovar 4b (CECT 4032) and serovar 1/2a, which was previously isolated in our laboratory from a fresh-cut lettuce sample.[21] *Salmonella* strains were grown individually in tryptone soy broth (TSB, Oxoid) for 20-24 h at 37 ± 1 °C. *L. monocytogenes* strains were grown individually in TSB supplemented with 6 g L^{-1} of yeast extract (tryptone yeast extract soy broth, TSBYE) for 20-24 h at 37 ± 1 °C. Bacterial cells were harvested by centrifugation at 9820 x g for 10 min at 10 °C and then re-suspended in sterile saline solution (SS; 8.5 g L^{-1} NaCl). Equal volumes of the four *S. enterica* concentrated suspensions were mixed to produce a single suspension, and equal volumes of the five *L. monocytogenes* concentrated suspensions were mixed to provide a second suspension. For each inoculum preparation, a volume of the concentrated bacterial suspension was added to saline peptone (SP; 8.5 g L^{-1} and 1 g L^{-1} peptone) to obtain approximately 10^6 CFU mL^{-1}. The inoculum concentration was checked by plating appropriate dilutions on Palcam agar for *L. monocytogenes* (Palcam Agar Base with selective supplement, Biokar Diagnostics) or on XLD (Xylose-Lysine-Desoxycholate Agar, Oxoid) for *S. enterica*. Plates were incubated at 37 °C for 24 h (*S. enterica*) or 48 h (*L. monocytogenes*).

Inoculation and storage

After the antioxidant treatment, twelve pear wedges were inoculated with *L. monocytogenes* by pipetting 15 µL of the bacterial suspension containing approximately 10^6 CFU mL^{-1} into the well of each wedge. In addition, twelve wedges were inoculated with 15 µL of the *S. enterica*

bacterial suspension. The pear wedges (approximately 110 g) treated with the antioxidant without pathogens plus one wedge inoculated with *S. enterica* or *L. monocytogenes* were placed in a polypropylene terephthalate tray, which was sealed with a non-peelable polypropylene terephthalate plastic film (APET-110, ILPRA, Italy) of 64 µm in thickness and O_2 permeability of 110 cm^3 m^{-2} day^{-1} atm^{-1} at 23 °C. There were 3 trays per pathogen for each recovery day.

Initially, samples were stored in conditions simulating a commercial application (constant storage at 4 ± 1 °C). Three trays per pathogen were examined at day 0 and three more after 3 days of storage at 4 ± 1 °C. Then, the rest of the samples were divided into two lots, one was stored at 8 ± 1 °C for 5 days (temperature abuse conditions) simulating more realistic conditions during transport and in a refrigerated display window while the other was maintained at a constant temperature of 4 ± 1 °C for 5 days.

Bacterial analysis

Recovery of pathogen populations were performed at day 0 and after 3 and 8 days of storage under the two conditions. Before opening the trays, the headspace gas composition was determined using a handheld gas analyser (CheckPoint O2/CO2, PBI Dansensor, Denmark).

To recover the pathogens from the wedges, a plug (1.2 cm in diameter and 1 cm deep, approximately 1 g $plug^{-1}$) containing the entire well was removed using a sterile cork borer. One plug per repetition was placed in a sterile filter bag (80 mL, BagPage®, Interscience BagSystem, Saint Nom, France) and diluted with 9 mL of buffered peptone water (BPW, Oxoid). The mixture was homogenized in paddle blender for 2 min at high speed (MiniMix, Interscience, France), and aliquots of the mixture were then serially diluted in SP and plated on XLD for enumerating *S. enterica* or on Palcam agar for *L. monocytogenes*. The agar plates were incubated at 37 ± 1 °C for 24 h (*S. enterica*) or 48 h (*L. monocytogenes*). The data were transformed to CFU g^{-1} pear. Three determinations per pathogen were made at each sampling point in duplicate.

Statistical analysis

All data were checked for significant differences by applying variance analysis (ANOVA) using the JMP8 (SAS, Statistical Analysis System) statistical package. They were subjected to mean separation by least significant differences by Tukey's test ($p< 0.05$).

RESULTS AND DISCUSSION

Several authors have reported that foodborne pathogens, including *L. monocytogenes* and *Salmonella* spp., may often be able to grow on the flesh of some fruits, including apples, peaches, strawberries, melons, watermelons, papayas, persimmons and pears.[22-29] The current study evaluated the behaviour of *L. monocytogenes* and *S. enterica* on minimally processed 'Conference' pears treated with an antioxidant solution and stored under MAP in conditions simulating commercial application (constant storage at 4 °C) and after a simulated cold chain break (temperature abuse conditions). The pear wedges used as a support matrix had SSC values from 14.5 to 14.8 °Brix (SSC average, 14.7 ± 0.1 °Brix, data not shown) and TA values between 2.29 and 2.80 g malic acid L^{-1} (TA average, 2.67 ± 0.22 g malic acid L^{-1}, data not shown). After 3 and 8 days of storage, the headspace gas composition of the packages was measured (Fig 1). In both storage conditions and regardless of the pathogen evaluated, a strong decrease in the O_2 levels was observed after 3 days of storage (approximately 9 kPa), reaching O_2 levels of 0 kPa at 8 days of storage. Regardless of the pathogen evaluated, after 3 days of storage a slight increase of CO_2 levels was observed with values of approximately 7 kPa of CO_2. After 8 days of storage, a significant difference between the storage conditions was observed, regardless of the pathogen evaluated. Samples stored under temperature abuse conditions reached higher concentrations of CO_2 (approximately 22 kPa) than samples under constant storage at 4 °C (approximately 18 kPa).

The initial populations of *L. monocytogenes* and *S. enterica* on fresh-cut pears were 3.2 ± 0.9 and 3.6 ± 0.3 log CFU g^{-1} pear, respectively (Fig. 2). After 3 days of storage at 4 °C, *L. monocytogenes* and *S. enterica* populations remained at the initial levels. After 8 days of storage, both constant storage (4 °C) and temperature abuse conditions caused a significant decrease of *S. enterica* populations on pear wedges, reaching 3.1 ± 0.2 and 3.0 ± 0.3 log CFU g^{-}

^{1} pear, respectively. In contrast, *L. monocytogenes* grew in both storage conditions. The values were 4.9 ± 0.5 log CFU g^{-1} pear (1.6 log increase) in samples stored at 4 °C and 5.4 ± 1.0 log CFU g^{-1} pear (2.20 log increase) in the temperature abuse conditions. Thus, the temperature increase for 5 days caused a greater increase in the *L. monocytogenes* populations on the fresh-cut pear product even in the highest CO_2 levels (22 kPa). Other researchers have observed that an active MAP containing 5 kPa O_2 and 30 kPa CO_2 had no inhibitory effect on the growth of *L. monocytogenes* on cactus-pear fruit at 4, 8, 12 and 20 °C,[30] or on coconut packaged under the same conditions and stored at 2, 4, 8 and 10 °C.[31] No significant differences between *L. monocytogenes* and *S. enterica* populations on fresh-cut pears were observed at the initial day or after 3 days of storage. However, significant differences among the pathogens were observed at 8 days in both storage conditions; the *L. monocytogenes* population on fresh-cut pears increased, whereas the *S. enterica* populations on fresh-cut pears decreased during same time. In fact, the effect of temperature in the growth rate of foodborne pathogens is well known. Alegre et al. [22] noticed that *Salmonella* and *L. innocua* were able to grow on fresh-cut apples stored at room temperature and were unaffected by MAP or the addition of antioxidants. Thus, the observed behaviour of *S. enterica* on fresh-cut pears in the present study could be due to the storage temperature (below 10 °C).

Previously, we observed that *L. monocytogenes* was able to grow on fresh-cut pears processed (without antioxidant treatment and without MAP) at different ripeness stages and that the growth rate of *L. monocytogenes* increased with increasing temperature.[24] Alegre et al. [22, 23] determined the survival and growth of *E. coli* O157:H7, *Salmonella* and *Listeria innocua* on some minimally processed fruits, including peaches and apples stored at different temperatures. The influence of fruit cultivar, use of antioxidant solution and passive MAP were also evaluated. Under refrigeration (5 °C), only *L. innocua* could grow on 'Elegant Lady' peach plugs and on 'Golden Delicious' apples. Nevertheless, in both matrices, *E. coli* O157:H7, *Salmonella* and *L. innocua* were able to grow on peach and apple plugs when incubated at 10 °C.

CONCLUSION

This is the first report that evaluates the behaviour of *S. enterica* and *L. monocytogenes* on minimally processed pears under conditions simulating commercial applications and a cold chain break. Although refrigeration at a constant temperature (4 °C) effectively inhibited the growth of *S. enterica* on fresh-cut pears, it did not affect the growth of *L. monocytogenes*.

This study demonstrated that *L. monocytogenes* was able to grow on fresh-cut pears processed under the conditions described here, despite low pH, refrigeration and use of modified atmosphere. Thus, minimally processed pears should be protected from contamination by this pathogen during preparation, and food business operators should guarantee the absence of *L. monocytogenes* in 25 g of fruit before the food has left its control area.

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Figure captions

Figure 1. The headspace gas composition of the packages of fresh-cut 'Conference' pears artificially inoculated with *L. monocytogenes* (open symbols) and *S. enterica* (full symbols) stored under constant storage temperature at 4 °C (continuous line) and under the temperature abuse conditions (dotted line) (n=6). Within each gas, difference ($p < 0.05$) among storage conditions and pathogens in each sampling point is represented by letters (a, b, c and d).

Figure 2. Populations of *L. monocytogenes* and *S. enterica* on fresh-cut 'Conference' pears stored in conditions simulating a commercial application (constant storage at 4 °C for 8 days) or in temperature abuse conditions (3 days at 4 °C plus 5 days at 8 °C). The data represent the mean of three determinations and two experimental repetitions (n=6). Different letters indicate significant differences ($p < 0.05$). Uppercase letters (A, B, C and D) represent differences among different sampling points in *L. monocytogenes* populations and lowercase letters (a, b, c and d) represent differences among different sampling points in *S. enterica* populations. Difference among pathogens in each sampling point is represented by * ($p < 0.05$).

Figure 1

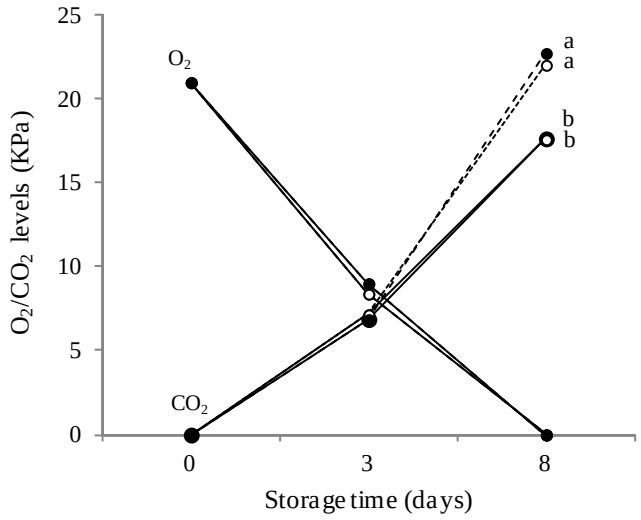

Figure 2