

Survival and growth of *Listeria* species in a model ready-to-use vegetable product containing raw and cooked ingredients as affected by storage temperature and acidification

Christopher Thomas*, Olive Prior & David O'Beirne

Food Science Research Centre, Department of Life Sciences, University of Limerick, Limerick, Ireland

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Summary The survival and growth of *Listeria monocytogenes* and *L. innocua* strains inoculated onto cooked sweet corn and fresh bean sprouts packed individually, and as components of a combination product, were examined at refrigeration and mild abuse temperatures. Growth rates were both temperature and vegetable dependent. Maximal growth rates ($1.14 \pm 0.11 \log$ CFU/day) were identified on cooked sweet corn at 12 °C. The inclusion of cooked sweet corn did not significantly increase ($P > 0.05$) the growth rate or final *Listeria* population density of bean sprouts stored at 8 °C and 12 °C. The sensory quality of bean sprouts was relatively temperature independent for the initial 48 h of storage, but was maximized (4 days shelf life) at 3 °C. Acidification of sweet corn to pH 5, particularly with citric acid, slowed *Listeria* growth and could be an additional hurdle to supplement temperature in maintaining the safety of minimally processed vegetable combination products.

Keywords Acidification, *Listeria monocytogenes*, mesophilic bacteria, modified atmosphere, packaging, vegetables.

Introduction

Fresh, convenient, ready-to-use foods including minimally processed vegetables (MPVs) are increasingly popular and currently represent a significant proportion of the food market (Reyes, 1996). Their scope normally includes washed, chopped, sliced or grated fresh raw vegetables and has diversified to incorporate cooked ingredients such as sweet corn and pasta.

Both spoilage and pathogenic populations have been identified within MPVs having implications for product shelf-life and consumer safety. Microbial spoilage processes involve successive colonisation by species largely consisting of

pseudomonads, lactic acid bacteria and Enterobacteriaceae during the early stages, whilst yeasts and moulds play an increasing role with continued storage. A large diversity of pathogenic micro-organisms has been isolated from MPVs. Pathogens including *Listeria monocytogenes* and *Clostridium botulinum* are naturally present in soils, and inevitably contaminate fresh produce (Beuchat & Ryu, 1997); outbreaks of foodborne disease involving enteric pathogens such as enteropathogenic *Escherichia coli* have also been documented (Merson *et al.*, 1976).

L. monocytogenes has been isolated from vegetables at frequencies varying from 0 to 48% (Lainé & Michard, 1988; Breer & Baumgartner, 1992; Gunasena *et al.*, 1995). The potential for listerial survival and growth at refrigeration temperatures has been identified on intact fresh pro-

Correspondent: Fax: +353 61 202572;
e-mail: chris.thomas@ul.ie

duce (Berrang *et al.*, 1989) and MPVs including minimally processed cabbage (Kallender *et al.*, 1991), endive (Carlin *et al.*, 1995) and broccoli (Berrang *et al.*, 1989). However, whilst the fate of contaminating listerial populations has been demonstrated to vary with vegetable type, processing procedures and storage temperature, inconsistencies within the data still remain.

Refrigeration, in addition to suppressing the growth of pathogens, retards the principal physiological deteriorative and microbial spoilage processes of MPVs (Reyes, 1996). However, risk of temperature abuse has prompted the food industry to search for additional mild preservation techniques. Modified atmosphere packaging (MAP) is being increasingly applied within preservation systems for many foods including MPVs. Passive MAP is regularly applied to MPVs such as stir fry mixes overwrapped with semi-permeable polyethylene films, harnessing the respiratory activity of living vegetable tissue to modify atmospheric conditions, extending the product's shelf life through a reduction in the rates of product respiration and enzymatic browning (O'Beirne, 1990).

The present investigation was undertaken to evaluate the significance of incorporating cooked components into a sealed MPV product with respect to the population dynamics of *Listeria* strains and the spoilage microflora at refrigeration and temperature abuse conditions.

Materials and methods

Preparation of model vegetable products

Sweetcorn (34 g) which had been cooked and canned with no preservatives was drained and weighed out into transparent plastic trays (180 × 130 × 25 mm). Raw bean sprouts (*Vigna radiata*) (66 g) were then added to the canned sweet corn and the combination product was overwrapped using a low migration catering cling film (Wrap Film Systems, Shropshire, England) and heat sealed. The film, with a thickness of 8–10 µm, was identified to have permeabilities to oxygen and carbon dioxide of 15000 and 90000 cm³ m⁻² d⁻¹ atm⁻² at 23 °C respectively. All products were stored at 3 °, 8 ° or 12 °C for 10 days and sampled regularly.

Listeria strains, growth and inocula preparation

L. monocytogenes serovars 4a (ATCC 19114) and 4b (NCTC 11994), and *L. innocua* serovar 6a (NCTC 11288) were maintained at –20 °C in nutrient broth No. 2 (Oxoid CM 67) supplemented with 15% (v/v) glycerol. Resuscitation was achieved by thawing the culture at room temperature (18–22 °C) and inoculation into nutrient broth No. 2 (Oxoid CM 1) and onto nutrient agar (Oxoid CM 3) followed by incubation at 35 °C for 24 h. Population density was assessed spectrophotometrically at 550 nm and adjusted to approximately 10⁸CFU/g prior to centrifugation (4000 g), washing, and re-suspension in sterile distilled water.

Product inoculation

Prior to overwrapping, test packs were inoculated with ten 10 µl aliquots of the prepared suspension which were distributed randomly over the product surface. The inoculated product was then overwrapped, gently shaken to assist inoculum distribution and transferred to the assigned storage conditions.

Microbiological analysis

Microbiological analysis was performed upon the individual components of both combination and single product (100g) packs. Samples (10g) were aseptically removed, diluted (1/10) with sterile peptone water (Oxoid CM 9) and homogenized with a stomacher lab blender (Model 400, A. J. Seward, London, UK). The prepared homogenates were decimally diluted in sterile peptone water and plated in duplicate on appropriate culture media.

All inoculated packs were assessed for *Listeria* populations by surface plating on *Listeria* selective agar [Oxford formulation (Oxoid CM856 + SR140); 35 °C for 48 h]. Non-inoculated controls were assessed for total aerobic mesophilic populations [plate count agar (Oxoid CM 325); 30 °C for 48 h] and Lactic acid bacteria [deMan Rogosa Sharpe agar (Oxoid CM 359) 30 °C for 72 h; in candle extinction jars (approximately 10% residual O₂)]. Listerial colony confirmation was per-

formed on characteristic black, aesculin producing colonies removed at each sample point using Gram stain, catalase, motility and biochemical tests [API Listeria system (Biomérieux)].

Initial rates of bacterial population growth under the experimental conditions were calculated from the gradient of the plot of log viable count against time (units of reciprocal time i.e. log CFU/day) over the first 4 days of the trial using Excel 7 for Windows 95.

Chemical and sensory analysis

At each sampling, the gaseous composition (oxygen and carbon dioxide) of the atmosphere within the packs was analysed using a Systech gas analyser (Gspace2; UK).

Visual spoilage of the bean sprout component of the products was assessed using a sensory panel consisting of 10 individuals. All panelists had previously been trained and vetted through evaluation of their grading of bean sprouts stored for different time periods. All sensory testing was performed under standard fluorescent lighting and against a blank white background. Visual spoilage of sweet corn was not assessed as previous experiments had demonstrated the inability of the panel to detect visual changes in sweet corn during storage (results not shown).

Triplicate measurements of the colour of the bean sprouts were also taken at each sample time using a Minolta chromameter (model 508i). The recorded parameters: L (lightness/darkness), a (greenness/redness), b (yellowness/blueness), were recorded and combined to calculate colour difference ΔE ($\sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$).

Effects of sweet corn acidification on *L. monocytogenes* and total mesophiles

To investigate the potential restriction of *L. monocytogenes* proliferation within the sweet corn component of mixed products, sweet corn was acidified by dipping for 1 h in two parts (w/v) citric or acetic acid solutions at 4 °C. Following dipping, the sweet corn was inoculated with *L. monocytogenes* ACTC 11994. It was then packaged with bean sprouts, overwrapped and stored at 8 °C for 10 days. *Listeria* and total mesophilic populations were assessed immediately following inoculation and at the completion of the trial as was the pH of sweet corn macerates (one part per two parts distilled water, w/v).

Statistical analysis

At each sample point duplicate estimations of microbial population density, nine replicate measurements of colour and one of gaseous composition and pH were taken. All experiments were performed in triplicate, and hence reported microbial populations represent the mean of six estimations etc. Statistical differences of the complete data sets were assessed using analysis of variance (Excel 7).

Results

The fate of *Listeria* spp. in individual and combination products

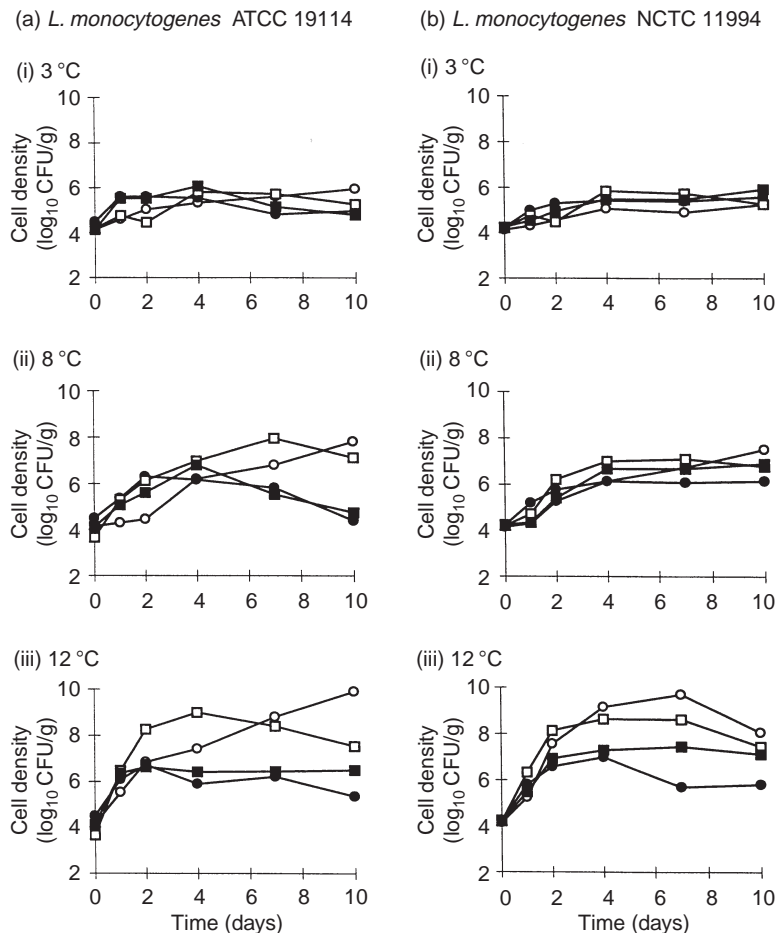
Growth rates of the three *Listeria* strains (Table 1) were not significantly different ($P < 0.05$) under each of the conditions examined and

Table 1 Growth rates of *Listeria* strains on the individual and combined components of a model RTU combination product over the initial 4 days of storage

	Growth rates (log CFU/day) in different vegetable products											
	Sweet corn			Bean sprouts			Sweet corn (mixed)			Bean sprouts (mixed)		
	3 °C	8 °C	12 °C	3 °C	8 °C	12 °C	3 °C	8 °C	12 °C	3 °C	8 °C	12 °C
<i>L. innocua</i>	0.19	0.63	1.00	0.23	0.56	0.35	0.14	0.60	1.09	0.19	0.46	0.38
<i>L. mono</i> 19114	0.29	0.59	0.80	0.21	0.41	0.29	0.38	0.78	1.26	0.42	0.65	0.47
<i>L. mono</i> 11994	0.23	0.53	1.3	0.27	0.45	0.64	0.38	0.74	1.07	0.31	0.65	0.75

Each result is the mean of three replicates all standard deviations were less than 3% of the mean.

Figure 1 Effects of storage temperature on the growth of (a) *L. monocytogenes* ATCC 19114 and (b) *L. monocytogenes* NCTC 11994 in individually packaged cooked sweet corn (○) and bean sprouts (●) and in these individual components when stored as a mixed product, sweet corn (□) and bean sprouts (■). Population represent the means of six replicate determinations. Standard deviations were lower than 0.3 log₁₀ CFU/g.



none of the experimental treatments resulted in a significant decrease ($P < 0.05$) in population density from initial levels (Figs 1a, 1b; Fig. 2a).

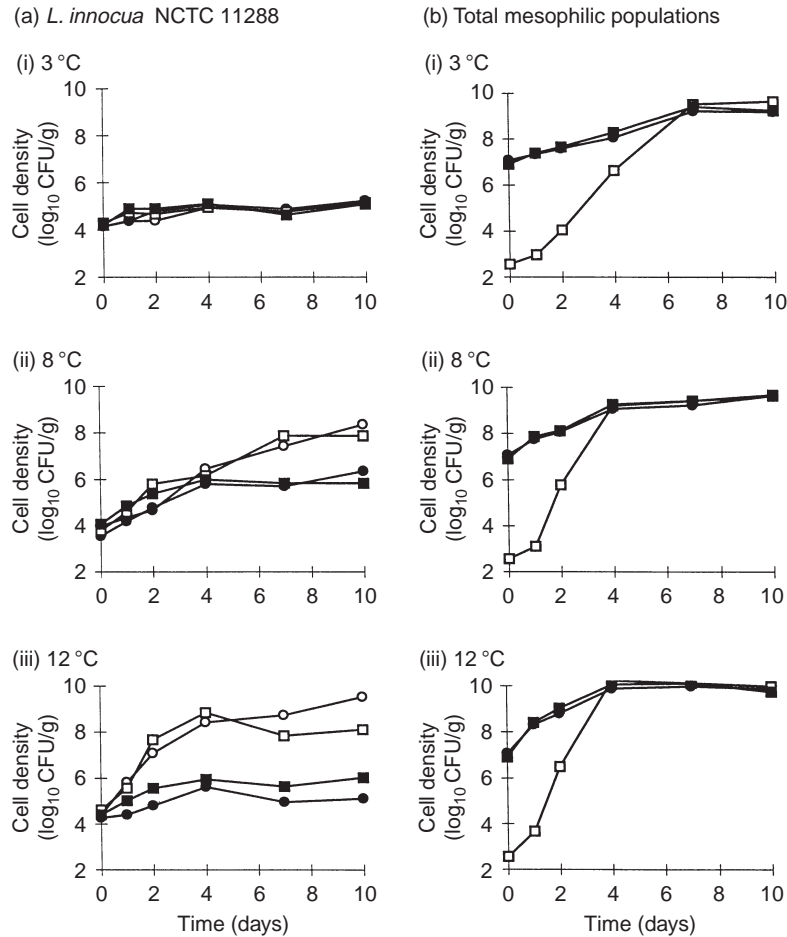
Storage at 3 °C proved to be listeristatic in all products with, approximating 10⁴ CFU/g persisting over the 10 day storage period and no significant differences ($P > 0.05$) between the final populations of the different strains. At mild abuse storage temperatures (8 °C and 12 °C), growth of the three strains was clearly evident in both individual and mixed product components. However, the time before populations increased significantly was both strain and product dependent. At 8 °C, regardless of the presence/absence of bean sprouts, *L. innocua* on sweet corn demonstrated a growth rate of approximately 0.6 log CFU/day over the first 4 days, acquiring and maintaining a density of 10^{7.5-8.0} CFU/g from day 7 onwards. However, despite growth rates comparable to

those on sweet corn over the initial 4 days of the trial (0.5 log CFU/day), *L. innocua* populations on bean sprouts were significantly lower ($P < 0.05$) than those on sweet corn by day 7. *L. monocytogenes* ATCC 19114 behaved similarly whilst no significant changes in population ($P > 0.05$) were detected with *L. monocytogenes* NCTC 11994 on the different products at any time.

At 12 °C, growth rates and population densities of *L. innocua* and *L. monocytogenes* ATCC 19114 on bean sprouts, regardless of the presence of sweet corn, did not increase significantly ($P > 0.05$) in comparison to those at 8 °C. By contrast, *L. monocytogenes* strain NCTC 11994 demonstrated a significant increase in initial growth rate in bean sprout-containing products with the increase in temperature (Table 1).

On sweet corn products held at 12 °C all

Figure 2 Effects of storage temperature on the growth of (a) *L. innocua* NCTC 11288 and (b) total mesophilic populations in individually packaged cooked sweet corn (○) (*L. innocua* only) and bean sprouts (●) and in mixed product, sweet corn (□) and bean sprouts (■). Population represent the means of six replicate determinations. Standard deviations were lower than 0.35 log₁₀ CFU/g.



Listeria strains behaved similarly, with increased mean initial growth rates of 0.45 and 0.43 log CFU/day respectively in individual and mixed product. Generally the growth rates and population density at day 10 (*Listeria* strains) were increased by the addition of sweet corn to bean sprouts, regardless of temperature. The magnitude of this difference increased directly with storage temperature.

The fate of total mesophilic populations

The total mesophilic populations on combination product components, packaged individually and as combined product, proliferated under all storage conditions (Fig. 2b). A final and maximum population exceeding 10⁹ CFU/g was observed within all products by day 4 at 8 °C and 12 °C and by day 7 at 3 °C. Colonisation of sweet corn by the indigenous mesophilic populations of bean

sprouts occurred rapidly at all temperatures with initial growth rates of 1.0, 1.7 and 2.0 log CFU/day at 3 °, 8 ° and 12 °C, respectively. The inclusion of sweet corn within mixed product was seen to have no significant effect ($P > 0.05$) on the initial growth rates or maximum populations observed on bean sprouts.

Gaseous changes

The concentrations of oxygen and carbon dioxide within the packs varied with temperature and the packaged product. As expected, no significant flux in oxygen and carbon dioxide concentrations was observed in packaged sweet corn at any temperature. However, in packs of products containing bean sprouts, both of which demonstrated similar characteristics, oxygen levels decreased to less than 3% after 4 days at abuse temperatures

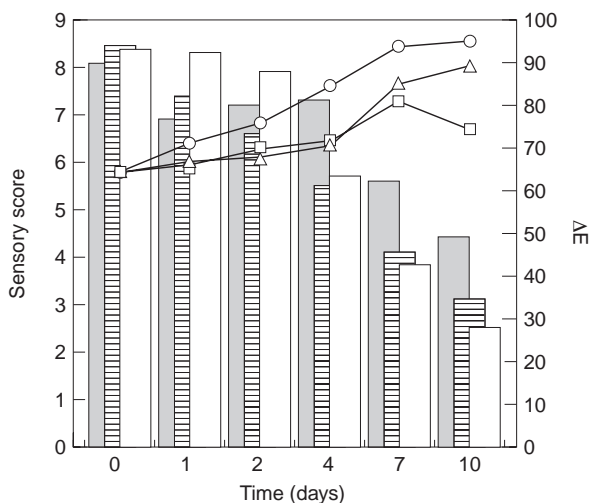


Figure 3 Changes in the sensory perception and total colour change (ΔE) of bean sprouts during storage at 3 °C (filled column; \square), 8 °C (striped column; \triangle) and 12 °C (white column; \circ). Sensory scores are the mean of 30 replicates; total colour change measurements are the means of nine replicates.

and remained at that level throughout the trial. Carbon dioxide levels increased to 5% and 12% at 8 °C and 12 °C respectively, equilibrating to 7% by day 7 until the end of the trial. Gas flux was much reduced in product stored at 3 °C, with oxygen levels decreasing to a minimum of 15% and carbon dioxide rising to a maximum of 2% at the end of the trial period (results not shown).

Sensory and colour changes

Changes in sensory perception (visual scores only) and total colour change (ΔE) for bean sprouts are illustrated in Fig. 3. No significant differences ($P > 0.05$) were found between the sensory scores for bean sprouts held at 12 °C prior to day 4 of the trial; however, decay set in rapidly after this. At 3 °C, significant decreases ($P < 0.05$) in the sensory score of the product were identified on days 1, 2, 4, 7 and 10 whilst at 8 °C significant decreases ($P < 0.05$) were identified on all sample days. By day 4 of the trial, sensory quality was optimal at 3 °C and remained that way for the remainder of the trial. There was no significant differences between ΔE for all temperatures for the initial 48 h of storage. After this point, ΔE values for bean sprouts stored at 12 °C were significantly greater

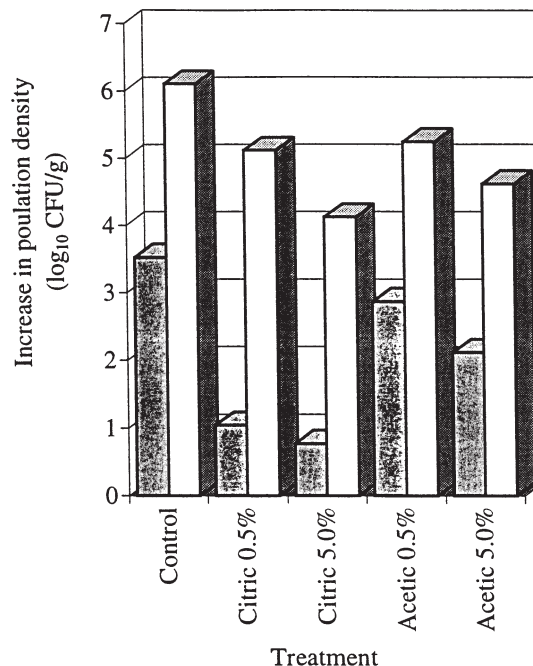


Figure 4 Changes in *L. monocytogenes* ATCC 19114 (filled) and total aerobic mesophilic populations (blank) of sweet corn dipped in citric or acetic acid at concentrations of 0.5 and 5.0% and mixed with bean sprouts during storage at 8 °C for 10 days.

than those for 8 °C and 3 °C. ΔE readings for 3 °C and 8 °C remained the same up to day 7; after this point values were significantly greater ($P < 0.05$) in bean sprouts stored at 8 °C. Examination of L (lightness), a (red/green) and b (yellow/blue) readings used to calculate ΔE identified the most significant changes to be in L values (results not shown). The inclusion of sweet corn did not prompt a significant change ($P < 0.05$) in the sensory grade or colour of bean sprouts in mixed products compared to bean sprouts packaged alone.

Impact of acidification on Listeria and total mesophilic populations

Acidification of cooked sweet corn with citric acid and acetic acid reduced the proliferation of *L. monocytogenes* ATCC 19114. Dipping the sweet corn in 5.0 and 0.5% citric acid and acetic acid resulted in post dipping pH values of 3.6, 5.2, 5.0 and 5.6 respectively in comparison to a pH of 7.2 in unacidified sweet corn.

Citric acid demonstrated increased Listeriostatic activity in comparison to acetic acid. Maximum restriction of both *Listeria* and mesophile populations was observed following dipping in 5.0% citric acid however, this was only significantly improved ($P < 0.05$) over that observed with 0.5% citric acid for the mesophilic populations.

Discussion

During these investigations, the two strains of *L. monocytogenes* generally behaved similarly on all products and temperatures, extending the observations made by Carlin *et al.* (1996a) on endive at 3 °C. The survival and growth patterns demonstrated that the characteristics of *L. innocua* are similar to those of the two strains of *L. monocytogenes*.

The growth rates of *Listeria* spp. on bean sprouts indicate that these products are largely unsuitable for listerial growth, possibly as a result of the limited nutrient availability of intact vegetables, as proposed by Carlin *et al.* (1996a). The organisms also encounter competition from the extensive indigenous microbial population of the product (10^7 CFU/g). Lactic acid bacteria populations, increased from 10^4 to 10^8 and 10^9 CFU/g at 3 °C and 12 °C, respectively (results not shown) and an isolate of *Leuconostoc* spp. was tentatively identified as the principal component. Members of this genus have previously been linked to the spoilage of grated carrots and bean sprouts (Carlin *et al.*, 1990; Varoquaux *et al.*, 1996).

The behaviour of *Listeria* spp. on bean sprouts was largely unaffected by the addition of sweet corn. However, the opportunity for transfer of microbial populations from bean sprouts to sweet corn was evident. The high availability of nutrients and absence of competitive microflora on cooked sweet corn can result in rapid growth of a contaminating population as evidenced by the growth of *Listeria* on sweet corn at 8 ° and 12 °C. Thus on a 50 : 50 bean sprout : sweet corn sample listerial growth extent would be up to 1.5 log higher than on bean sprouts alone.

In accord with Kallender *et al.* (1991) and Carlin *et al.* (1996b) growth of *Listeria* was unaffected by the elevated carbon dioxide and reduced

oxygen concentrations resulting from the combined respiratory activity of the bean sprouts and the high initial microbial population.

The major symptoms of bean sprout deterioration are defined as the darkening of sprouts and the development of sliminess and musty flavours (Lipton *et al.*, 1981). Using a sensory evaluation panel, a cut off score of 6 was used to determine the end of useful shelf life. The shelf life of bean sprouts was relatively temperature independent over the initial days of the trial, an observation supported by the stability of ΔE over this period. The high sensory rating of bean sprouts stored at 12 °C is perhaps the result of the rapid accumulation of carbon dioxide reducing respiration rate and delaying senescence, as proposed by Kader (1980). However, sensory acceptability is extended to at least day 4 by storage at 3 °C in agreement with previous shelf life estimates (Lipton *et al.*, 1981; Varoquaux *et al.*, 1996).

Abuse temperatures approximating 8 ° and 12 °C are not uncommon during commercial preparation and transport (Scandella *et al.*, 1990). When the growth potential of *Listeria* under these conditions is considered, and particularly on sweet corn, the absence of visual spoilage over the initial storage period has important implications for the safety of combination products where refrigeration is the sole hurdle.

Inclusion of an additional hurdle in the acidification step was beneficial in retarding the growth of *L. monocytogenes*. Acidification with citric acid (0.5%; pH 5.2) was seen to approximate the inhibition of *L. monocytogenes* achieved with higher concentrations. Citric acid was more inhibitory to the strain investigated than acetic acid, in agreement with Nguyen-the *et al.* (1996) but conflicting with studies in culture media (Young & Foegeding, 1993). Nguyen-the *et al.* (1996) further identified citric acid to be more suitable than acetic acid for application to sweet corn combined with green leaf vegetables (endive) as a result of it not migrating on to or causing necrosis of leaves.

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