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ORIGINAL ARTICLE

# Growth and survival of *Shigella sonnei* and *S. flexneri* in minimal processed vegetables packed under equilibrium modified atmosphere and stored at 7°C and 12°C

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*The growth and survival of* Shigella flexneri *and* Shigella sonnei *at 7°C and 12°C was evaluated in culture media (BHI and LB) and in three minimal processed vegetables (mixed lettuce, grated carrots and chopped bell peppers) packed in optimized EMA packages. In BHI and LB growth was observed at 12°C for* Shigella *sp. and both species survived at 7°C although it was noted that* S. flexneri *was more sensitive to temperature reduction. Growth and survival in EMA-packaged vegetables stored at 7°C and 12°C was variable depending upon the type of vegetable mix used. In the mixed lettuce the total aerobic plate count dominated spoilage and* Shigella *sp. maintained high numbers (*$\geq 10^5$ *cfu* $g^{-1}$*) throughout the shelf life (7 days). In grated carrots and in chopped bell peppers spoilage was dominated by lactic acid bacteria leading to a pH drop and a gradual die-off of the inoculum after 7 days of storage but low numbers of* Shigella *sp. may still be present (*$< 10^2$*–*$10^4$ *cfu* $g^{-1}$*) and in view of the low infective dose of the pathogen they may pose a hazard.* 

## Introduction

Each year there are a significant number of outbreaks of shigellosis from consumption of contaminated foods (Todd 1997, Mead et al. 1999). Unlike other bacterial foodborne pathogens, such as *Salmonella*, *Shigella* spp. are not associated with any particular food. The contamination of foods with *Shigella* usually results from an infected food handler after the food is processed because of improper preparation techniques (Lampel et al. 2000). Imported foods from endemic regions where hygienic standards are insufficient have become potential sources of contaminated foods. The source of many shigellosis outbreaks have been traced to the ingestion of raw or fresh vegetables. In 1992, an outbreak of *S. flexneri* in Michigan was linked to a tossed salad (Dunn et al. 1995). Iceberg lettuce was the incriminated food in an outbreak of *S. sonnei* in 1994 in a few European countries (Kapperud et al. 1995). During 1995, outbreak investigations in the United States linked *Shigella* spp. to lettuce and fresh green

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onions (Beuchat 1996, Tauxe et al. 1997). Fresh parsley was associated with an outbreak of *S. sonnei* in the United States and Canada in 1998 (CDC 1999).

Advances in agronomics, processing, preservation and distribution technologies have enabled the fresh produce industry to supply the consumer with nearly all types of high-quality fresh vegetables the year around. Equilibrium-modified atmosphere (EMA) packaging is nowadays widely applied to package fresh-cut produce (Day 1990). In this preservation technique, the air around the commodity is altered by a gas combination of 1–5% $O_2$ and 5–10% $CO_2$ (compensated with nitrogen). This atmosphere is established within hermetically sealed packages as a consequence of produce respiration. If produce respiratory characteristics are properly matched to film permeability values then a beneficial EMA can be reached (Carlin et al. 1990, Exama et al. 1993). The shelf life of the vegetables stored under EMA is extended 50% or more compared with air-stored vegetables (Jacxsens et al. 1999).

Changes in preservation technology creates new challenges for assuring safety aspects of the product. *Shigellae* were believed to be fragile and not particularly resistant to environmental stress. However, *Shigella* sp. survived for several days at both ambient temperature and refrigerator temperatures when inoculated onto various commercially prepared salads and vegetables (Rafii et al. 1995, Rafii and Lunsford, 1997) and grew readily in a wide range of foods even at 12°C (Zaika and Scullen 1996). The objective of this study was to evaluate the growth and survival of *S. flexneri* and *S. sonnei* first in optimum conditions *in vitro* and afterwards on three minimal processed vegetables packed in optimized EMA packages (a recently introduced packaging technology of fresh produce) stored at both 7°C and 12°C.

## Material and Methods

### *Bacterial strains*

*S. sonnei* CIP8249 and *S. flexneri* CIP8248 were obtained from the Institut Pasteur—Paris (France). Stock cultures of the two strains were maintained on Trypton Soy Agar (TSA; Oxoid, Basingstoke, England) slants at 4°C.

### *Growth/survival of* Shigella *sp. in broth at 7°C and 12°C*

The experiment was performed in order to evaluate the growth characteristics of the two *Shigella* species at a reduced temperature in an optimum medium, although with different nutrient contents, i.e. Brain Heart Infusion broth (BHI, Oxoïd), a nutrient-rich culture medium, and Luria Bertani broth (LB; NaCl $10{\cdot}0\ g\,l^{-1}$, pancreatic digest of casein $10{\cdot}0\ g\,l^{-1}$, yeast extract $5{\cdot}0\ g\,l^{-1}$, pH 7·0), a nutrient-poor culture medium, and to determine the effect of enumeration on a selective medium, HektoEn Agar (HEA, Oxoïd), compared to a non-selective medium, TSA on the recovery of the *Shigella* species.

From a stock culture, a subculture was placed in BHI and incubated for 18–20 h at 37°C. Appropriate dilutions in peptone-saline solution ($8{\cdot}5\ g\,l^{-1}$ NaCl, $1\ g\,l^{-1}$ peptone) of the overnight BHI culture were inoculated in BHI and LB in order to obtain an inoculum level of *c.* $10^5$ cfu $ml^{-1}$. Separate inocula were prepared for *S. flexneri* and *S. sonnei*. The inoculated BHI and LB was incubated at both 7°C and 12°C for 7 days and at selected time intervals the bacteria were enumerated on both TSA and HEA (plates were incubated at 37°C for 24 h). Tests were performed in duplicate.

### *Growth/survival of* Shigella *sp. in EMA-packaged minimal processed vegetables stored at 7°C and 12°C*

*EMA packaging of vegetables.* Mixed lettuce (a mixture of endive (*Cichorium endivia* L.), lollo rosso and lollo bionta (*Lactuca sativa* var. *crispa* L. (red and green variety)) and radicchio (*Cichorium intybus* var. *foliosum* L.) and chopped bell peppers (a mixture of green, red and yellow shredded bell peppers (*Capsicum annuum* L.) were prepared industrially in a local vegetable processing industry. Carrots (*Daucus carota* L.) were obtained freshly from the vegetable auction and peeled and hand-cut in sticks of

$3 \times 3 \times 40\,mm^3$ with a kitchen cutter (Philips, the Netherlands). For mixed lettuce portions of 250 g were weighed, for chopped bell peppers and shredded carrots portions of 150 g were weighed into bags made up of a selective film for $O_2$ and $CO_2$. The required film permeability for $O_2$ and for $CO_2$ of the plastic film to reach an equilibrium gas composition inside the package was calculated as indicated by Jacxsens et al. (1999). The packaging films used (Hyplast N.V., Hoogstraten, Belgium) were experimental films with a very high permeability for $CO_2$ at 7°C and 90% relative humidity (RH) ($>20\,000$ ml $CO_2$ $m^{-2}$ 24 h atm at 7°C and 90% RH). The films were selected only based on their $O_2$ permeability (at 7°C, 90% RH): PS 3 WA4862 film (30 µm, $PO_2 = 2023$ ml $O_2\,m^{-2}$ 24 h atm) to package the salad mix; PS1 WA 4060 film (30 µm, $PO_2 = 3700$ ml $O_2\,m^{-2}$ 24 h atm) to package shredded carrots; PS1 WA 4990 film (40 µm, $PO_2 = 2987$ ml $O_2/m^2$ 24 h atm) to package green–red–yellow paprika cubes mix (Jacxsens et al. 1999, 2000). Each portion was inoculated with appropriate dilutions of a BHI culture (18 h at 37°C) of *Shigella* to obtain a contamination level of *c.* $5 \times 10^5$ cfu $g^{-1}$ vegetable. This high inoculation level was applied although not always corresponding to the *Shigella* contamination in practice, because it enabled follow-up of the *Shigella* population by enumeration also in case a reduction was established. The vegetables were sprayed with the appropriate *Shigella* dilution in peptone-saline solution (PPS; 8·5 g NaCl $l^{-1}$ and 1 g peptone $l^{-1}$) applying a micropipette (Finpipette (40–200 µl), Labsystem, Helsinki, Finland) and gently mixed to have a homogenous distribution of the inoculated *Shigella* strain. Separate inocula were used for *S. flexneri* and *S. sonnei*. Appropriate dilutions of the BHI culture in PPS were plated on TSA to determine the exact inoculum. The required atmosphere (3% $O_2$, 5–10% $CO_2$, compensated with $N_2$) was introduced in the packages by means of a gaspackaging unit (gas mixer, WITT M618-3MSO, Gasetechnik, Germany; gas packaging, Multivac A300/42 Hagenmüller KG, Wolfertschwenden, Germany). Air products (Vilvoorde, Belgium) supplied the gases (Fresh line). At day 0 and day 7, a 40 ml gas sample was taken out of the head space of the packages with a syringe and injected into a $CO_2/O_2$ gas analyser (Servomex Food Package Analyser Series 1400, UK) to confirm the composition of the gas mixture in the packages. The pH was measured at day 0 and day 7 (for chopped bell peppers also on day 3 and day 5) by an electrode (PH 915600, Orion, Boston, USA) and measure unit (model 525A, Ankersmit, Boston, USA).

*Microbiological analysis of* Shigella *sp.* The inoculated EMA packaged minimal processed vegetables were stored at 7°C and 12°C for 7 days. Although 4°C is the optimum temperature for storage of EMA-packed vegetables, the maximum refrigeration temperature for storage of EMA packed vegetables in the distribution according to the Belgian legislation is 7°C. Therefore, the experiment was performed at 7°C (maximum refrigeration temperature in distribution) and 12°C (representing temperature abuse and supporting growth of *Shigella* spp.). The permeability of the EMA-packaging films were calculated for storage at 7°C. At regular time intervals two portions were taken, 10-fold diluted with peptone-saline solution, homogenized and *Shigella* sp. were enumerated by plating appropriate dilutions on HEA. HEA was incubated for 24°C at 37°C and typical colonies on HEA were streaked for purity on Nutrient Agar (NA; Oxoid) and further identified by API 20E (bioMérieux, Marcy-l'Etoile, France). At each sampling day, spoilage organisms were enumerated using the following media and incubation conditions: Plate Count Agar (PCA, Oxoid) for Total Aerobic Plate Count (TAC), pour plated and incubated at 30°C for 3 days; de Man-Rogosa-Sharp medium (MRS, Oxoid) for lactic acid bacteria (LAB), pour plated, overlaid with the same medium and incubated aerobically at 30°C for 3 days: Oxytetracycline Glucose Agar (OGA, Diagnostics Pasteur, Marnes-La-Coquette, France) + Oxytetracycline supplement (Oxoid) for yeasts, spread plated and incubated for 3 days at 30°C. All experiments were performed in duplicate (two packages per analysing time) and two samples from each package were analysed. Thus, each microbiological count is the mean of four analyses. The microbiological counts were statistically analysed by Microsoft Office Excel 97.

## Results and Discussion

### *Growth/survival of* Shigella *sp. in broth at 7°C and 12°C*

Similar growth in BHI and LB incubated at 12°C was observed for *S. sonnei* and *S. flexneri* (*c.* 2 log units increase in 2–3 days to obtain a maximum number of 7·5 log cfu $ml^{-1}$) (Fig. 1). The USDA Pathogen Modeling Program Version 5.1 (Buchanan and Whiting USDA, Wyndmoor, Pennsylvania, USA) indicates a mean generation time of 10·3 h for *S. flexneri* at 12°C pH 7·0 and 0·5% NaCl and thus predicts a 2 log increase in *c.* 3 days which is in agreement with the present results. At 7°C, no significant outgrowth was obtained but *Shigella* sp. survived both in BHI and LB. Similar to slightly higher numbers (*c.* 0·5 log unit) than the initial inoculum were present after 3 days at 7°C but after 7 days, a small reduction was noticed (*c.* 0·5 log unit in LB and *c.* 1·0 log unit in BHI) if compared to contamination at Day 3 (Fig. 1). The minimum temperature for growth in laboratory media was reported 6°C for *S. sonnei* (19/21 strains) and 7°C for *S. flexneri* (11/21 strains) (ICMSF 1996). It was noticed in the present experiment that in general, *S. flexneri* is more sensitive to temperature reduction (slower outgrowth at 12°C, reduced survival at 7°C) than *S. sonnei*. It is known that *S. flexneri* is rather more sensitive than *S. sonnei* to inhibiting factors such as reduced temperature, increased NaCl concentrations and nitrite (ICMSF 1996). The selectivity of the medium used for enumeration has an effect on the recovery of the *Shigella* sp. if they are stressed by prolonged storage at reduced temperatures. After 5 and 7 days at 7°C lower numbers were enumerated on HEA than on TSA especially for *S. flexneri* (Fig. 2). It is established that HEA is a high selective medium inhibiting growth of many non-shigellae, however, also affecting recovery of stressed *Shigella* sp. (Uyttendaele et al. 2001). It was established by Uyttendaele et al. (2001) that when testing a range of *S. sonnei* and *S. flexneri* strains including the above-mentioned CIP strains and a number of clinical isolates that these two CIP strains can be classified as resistant to acid and chill stress. The use of these two CIP strains for further challenge testing in EMA-packed vegetables thus represents a 'worst case' scenario for growth and survival of *Shigella* spp. in EMA-packed vegetables.

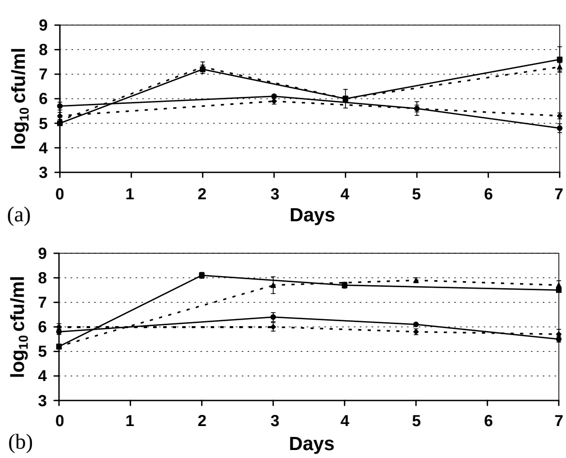


**Figure 1.** Effect of incubation temperature and incubation medium on growth/survival of (a) *S. flexneri* and (b) *S. sonnei* (enumeration on TSA) (BHI 12°C (—■—); LB 12°C (- - - -▲- - - -); BHI 7°C (—●—); LB 7°C (- - - -◆- - - -).

*Growth/survival of* Shigella *sp. in EMA-packed vegetables stored at 7°C and 12°C.* Growth and survival of *Shigella* sp. depended upon the type of vegetable mix used in the experimentation (Fig. 3). In the mixed lettuce (Fig. 3(a)) a multiplication of *Shigella* sp. was observed during storage at 12°C g (maximum one log unit increase). At 7°C survival of both *Shigella* species was noticed: variable counts within 1 log unit of the inoculum. The fluctuation in bacterial count per gram may reflect heterogenous distribution of the *Shigella* sp. on the various segments of the salad mix. The applied modified atmosphere in the mixed lettuce EMA

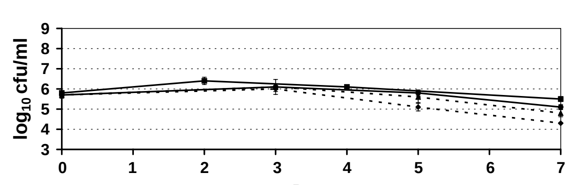


**Figure 2.** Effect of enumeration medium on recovery of *S. flexneri* and *S. sonnei* from BHI incubated at 7°C (*S. sonnei* TSA (—■—); *S. flexneri* TSA (- - - -▲- - - -); *S. sonnei* HEA (—●—); *S. flexneri* HEA (- - - -◆- - - -).

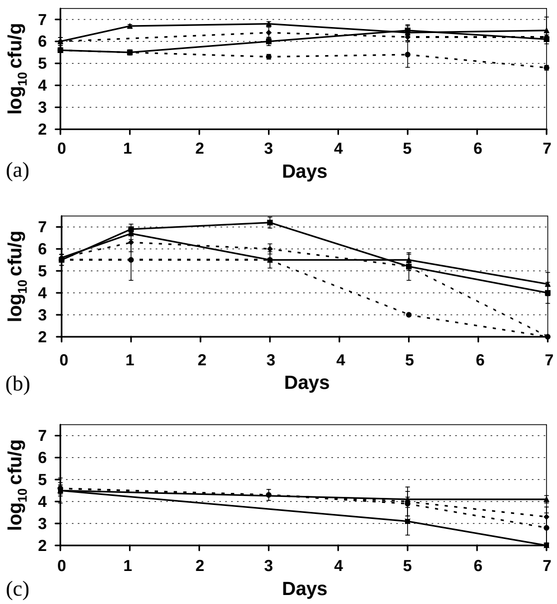


**Figure 3.** Growth/survival of *S. flexneri* and *S. sonnei* on EMA-packaged minimal processed vegetables (a) mixed lettuce, (b) grated carrots, (c) red/green/yellow chopped bell peppers (*S. sonnei* 12°C (—■—); *S. flexneri* 12°C (—▲—); *S. sonnei* 7°C (- - - -●- - - -); *S. flexneri* 7°C (- - - -◆- - - -) (detection limit $10^2$ cfu $ml^{-1}$).

packages (3% $O_2$ and 5% $CO_2$) (Table 1) did not affect in a direct way the growth/survival of the pathogen. According to Jacxsens (2002) the accumulated $CO_2$ concentration inside the mixed lettuce EMA package was too low to act as an antimicrobial agent. In the present experiment an almost anoxic atmosphere was established at Day 7 at 12°C because the oxygen permeability of the applied packaging film was designed for storage at 7°C and if exposed to 12°C (temperature abuse), respiration and thus oxygen consumption is increased and not longer equilibrated by diffusing oxygen from the air environment in which the EMA-packages are stored through the packaging film. Research performed by Jacxsens (2002) showed that application of EMA in combination with a good temperature control decreases the respiration rate of the package produce significantly, which causes longer retention of the typical stiffness of the plant tissue. This rigidity retention results in less leakage of nutrients and can be postulated as the reason for growth retardation of spoilage micro-organisms in EMA-packaged vegetables and subsequently prolongation of shelf-life (Jacxsens et al. 1999). In the present study spoilage of mixed lettuce was dominated by the TAC and higher numbers were obtained at 12°C than at 7°C (Table 1). This confirms reports of Jacxsens et al. (2002) determining the shelf life of EMA-packed mixed lettuce. These authors also mention that the genus *Pseudomonas* was most frequently isolated among bacteria in samples of lettuce and other minimal processed leafy vegetables.

In grated carrots (Fig. 3(b)) survival of *S. sonnei* at 7°C and significant outgrowth (*c.* 1·6 log unit) at 12°C was observed during the first 3 days but there was a 2 log decrease in the subsequent 2 days, both at 7°C and 12°C. *S. flexneri* showed little outgrowth at 12°C but survival at 7°C during the first 5 days of storage whereafter die-off was noticed. It had been demonstrated previously that although the intrinsic parameters of carrots could enable growth of *Shigella*, growth of *S. flexneri* was not observed in carrots at 28°C and 19°C (Zaika and Scullen 1996). Ethanolic extracts of peeled and shredded carrots have antimicrobial activity against several foodborne bacteria and yeast (Babic et al. 1994). These could also be partly responsible for the die-off noticed in the present experiment. However, as shown in Table 1, a rapid growth of LAB and yeasts was noted in shredded carrots during storage resulting in a pH drop (from pH 6·2 at day 0 to pH 5·0–5·1 at day 7). The competition with LAB is probably the major factor affecting the survival of *Shigella* sp. Jaxcsens et al. (1999) showed that EMA conditions favoured growth of LAB in shredded carrots and that by the end of shelf-life LAB dominated the TAC. Carlin et al. (1989) found that *Leuconostoc mesenteroides*, a heterofermentative LAB, comprises the main part of the spoilage organisms in shredded carrots. Because of the high initial contamination with LAB in the present study high numbers of LAB were already obtained on the EMA-packed shredded carrots after 3 days of storage at 7°C and 12°C (Table 1). Also from Day 3 on a die-off of *Shigella* sp. on EMA-packed shredded carrots was noted. As shown by Jaxcsens et al. (1999) the growth of LAB will cause less off-flavour compared to Gram-

**Table 1.** Changes (log cfu $g^{-1} \pm 95\%$ confidence interval) in total aerobic plate count (TAC), lactic acid bacteria (LAB), yeasts, pH and gas composition on EMA-packed mixed lettuce, grated carrots and chopped bell peppers stored at 7°C and 12°C

| Vegetable type | Temp. | Parameter | Day 0 | Day 1 | Day 3 | Day 5 | Day 7 |
|---|---|---|---|---|---|---|---|
| Mixed lettuce | 7°C | TAC | 6·4±0·2 | N.D. | N.D. | 7·6±0·3 | 9·1± 1·0 |
| | | LAB | 5·2±0·2 | N.D. | 5·4±0·3 | 6·2±0·1 | 7·0±0·3 |
| | | Yeast | 5·6±0·3 | N.D. | 5·2±0·2 | 5·3±0·2 | 6·8±0·2 |
| | | pH | 5·9 | N.D. | N.D. | N.D. | 6·2 |
| | | Gas comp. | 3% $O_2$–5% $CO_2$ | N.D. | N.D. | N.D. | 2% $O_2$–4% $CO_2$ |
| | 12°C | TAC | 6·4±0·2 | 6·8±0·1 | N.D. | 8·8±0·8 | 9·7±0·6 |
| | | LAB | 5·2±0·2 | 5·5±0·2 | 5·7±0·2 | 6·8±0·3 | N.D. |
| | | Yeast | 5·6±0·3 | 5·3±0·4 | 5·5±0·4 | 6·2±0·2 | N.D. |
| | | pH | 5·9 | N.D. | N.D. | N.D. | 6·1 |
| | | Gas comp | 3% $O_2$–5% $CO_2$ | N.D. | N.D. | N.D. | 0·1% $O_2$–6% $CO_2$ |
| Grated carrots | 7°C | TAC | 5·8±0·1 | 7·3±0·6 | 9·2±0·5 | 8·7±0·7 | 9·6±0·5 |
| | | LAB | 5·2±0·4 | 7·3±0·5 | 9·2±0·5 | 8·5±0·5 | 9·5±1·1 |
| | | Yeast | 3·9±0·5 | 4·3±0·2 | 6·9±0·2 | 6·5±0·5 | 7·3±0·1 |
| | | pH | 6·2 | N.D. | N.D. | N.D. | 5·1 |
| | | Gas comp | 2·8% $O_2$–3·4% $CO_2$ | N.D. | N.D. | N.D. | 4·5% $O_2$–5·5% $CO_2$ |
| | 12°C | TAC | 5·8±0·1 | 7·9±0·3 | 9·9±0·3 | 8·9±0·4 | 8·5±0·2 |
| | | LAB | 5·2±0·4 | 7·8±0·3 | 10·0±0·1 | 8·8±0·5 | 8·4±0·2 |
| | | Yeast | 3·9±0·5 | 5·1±0·3 | 7·0±0·2 | 6·2±0·9 | 6·6±0·4 |
| | | pH | 6·2 | N.D. | N.D. | N.D. | 5·0 |
| | | Gas comp | 2·8% $O_2$–3·4% $CO_2$ | N.D. | N.D. | N.D. | 6·0% $O_2$–9·3% $CO_2$ |
| Chopped bell peppers | 7°C | TAC | 5·5±0·2 | N.D. | 8·4±0·5 | 8·4±0·2 | 8·2±0·5 |
| | | LAB | 4·8±0·1 | N.D. | 6·1±0·3 | 8·3±0·2 | 8·1±0·2 |
| | | Yeast | 3·5±0·1 | N.D. | 6·4±0·4 | 6·2±0·2 | 6·7±0·3 |
| | | pH | 4·7 | N.D. | 4·5 | 4·1 | 4·0 |
| | | Gas comp | 3·1% $O_2$–5.3% $CO_2$ | N.D. | N.D. | N.D. | 8·3%$O_2$–23·8% $CO_2$ |
| | 12°C | TAC | 6·5±0·1 | N.D. | 9·3±0·2 | 8·8±0·3 | 8·6 |
| | | LAB | 3·5±0·6 | N.D. | 7·6±0·8 | 8·1±0·8 | 8·0±0·4 |
| | | Yeast | 4·7±0.4 | N.D. | 6·7±0·2 | 7·7±0·1 | 7·9±0·8 |
| | | pH | 5·1 | N.D. | 4·7 | 4·1 | 4·2 |
| | | Gas Comp | 2·9% $O_2$–5·1% $CO_2$ | N.D. | N.D. | N.D. | 8·6%$O_2$–14·7% $CO_2$ |

N.D. is not determined.

negative spoilage bacteria, however, at the high numbers (>9·0 log units) obtained in the present study a strong acetic odour was noted.

In the chopped bell peppers (Fig. 3(c)) no outgrowth of *Shigella* sp. was noticed. This could be ascribed to the acid pH of the product (pH 4·9±0·2 at day 0). The minimum pH for growth under optimum conditions is 4·9 (ICMSF 1996). As can be seen from Table 1 high numbers of LAB and yeasts were noted from day 3 on resulting in a pH drop to pH 4·1±0·1 at day 7. Jacxsens et al. (2002) reported that bell peppers contain more sugars compared to lettuce, which stimulates growth of LAB and yeasts. An increase of $CO_2$ until 14–24% inside the package on day 7 (Table 1) could also be explained by the development of heterofermentative LAB. These $CO_2$ concentrations are above the $CO_2$-tolerance limit of the plant tissue (10–15%) (Jacxsens et al. 1999) causing a die-off of the plant tissue and thus stopping respiration and thus $O_2$ consumption which explains the higher $O_2$ concentrations (*c.* 8%) inside the package than would be expected from an EMA-package (2–3%) (Jacxsens et al. 1999). As can be seen from Fig. 3(c) a significant reduction of *Shigella* on the EMA-packed chopped bell peppers was obtained after 7 days of storage. *S. sonnei* was shown to be the most sensitive strain (after 5 days at 12°C a 1·5 log reduction was noted). Rafii and Lunsford (1997) reported that in a crab salad with pH 4·4–4·5 stored at 4°C the count of *S. flexneri* had decreased about 1 log unit by day 8 and stayed approximately the same until day 20. Apart from acid pH and an effect of the competetive flora, antimicrobial components in the chopped bell peppers might have contributed to the die-off of *Shigella* sp noticed in the present experiment. Growth inhibition of some foodborne pathogenic bacteria by *Capsicum annum* extracts was reported by Dorantes et al. (2000). They found that some capsaicinoids and their precursors present in the *C. annum* extract possessed antimicrobial activity.

Other studies reported the survival of *S. flexneri* on various commercially prepared and vacuum packed vegetables. Rafii et al. (1995) reported survival of *S. flexneri* up to 10 days at 5°C and 10°C for carrots, cauliflower, celery, radish, and broccoli and Rafii and Lunsford (1997) reported survival of *S. flexneri* up to 12 days at 4°C for green pepper and onion and up to 26 days at 4°C for cabbage. However, in the studies by these researchers, the numbers had declined during storage at refrigeration depending upon the type of vegetable. For green pepper and onion the initial numbers were $5.2 \times 10^6$ and $5{\cdot}1 \times 10^6$ cfu g$^{-1}$, respectively, whereas after 12 days at 4°C they had declined to $2{\cdot}2 \times 10^4$ and $2{\cdot}1 \times 10^5$ cfu g$^{-1}$, respectively (Rafii and Lunsford 1997). Satchell et al. (1990) noticed survival of *S. sonnei* in shredded cabbage and no reduction in numbers if the shredded cabbage was packaged either under vacuum or modified atmosphere (30% $N_2$–70% $CO_2$) and stored under refrigeration for 7 days whereas under aerobic conditions the pathogen reduced to undetectable levels ($<1$ cfu g$^{-1}$) after 7 days at 0–6°C (initial inoculum was $6{\cdot}3 \times 10^2$ cfu g$^{-1}$).

Zaika and Scullen (1996) reported no growth of *S. flexneri* in canned vegetable broth (pH 5·48, 1·13% NaCl) at 12°C, which confirmed the prediction by the models they had developed, and they observed a gradual decrease of the pathogen population in the vegetable broth. In peas (pH 6·51, 0% NaCl), however, the same authors reported that the observed growth of *S. flexneri* at 15°C was much faster than predicted (Zaika and Scullen 1996). This indicates that apart from pH, $A_w$ and %NaCl (the parameters taken into account in the model) other food constituents can influence the growth kinetics. In the present experiment, growth was observed at 12°C for *Shigella* sp. and both species survived well at 7°C in BHI and LB. However, in addition to storage temperature and pH, growth and survival of *Shigella* sp. in the minimal processed vegetables was also influenced by the presence of a competitive flora (dominated by Gram-negative bacteria in the EMA-packed mixed lettuce and by lactic acid bacteria in the EMA-packed grated carrots and chopped bell peppers (Table 1)) and possibly natural antimicrobial components present in grated carrots (Babic et al. 1994) and chopped bell peppers (Dorantes et al. 2000). Therefore, it is recommended to use predicted models as an indication of growth kinetics but to confirm the safety of the product by challenge testing. In the present study, challenge testing of EMA-packaged vegetables showed that *S. sonnei* and *S. flexneri* survive in these minimal processed foods throughout their shelf-life. The low infective dose of *Shigella* sp. and the lack of lethal treatment during production, processing or preparation makes these products a serious hazard for shigellosis. The growing popularity of minimally processed fresh produce and the increased global trade exposing consumers to exotic microflora warrants the elaboration of prevention strategies. The contamination of foods usually occurs by irrigation of crops with water polluted with human faeces or by a food handler who did not take proper precautions such as hand-washing (Lampel et al. 2000). Proper sanitation is crucial at all levels in the fresh produce chain (Tauxe et al. 1997).

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