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The effect of micro-architectural structure of cabbage substratum and or background bacterial flora on the growth of *Listeria monocytogenes*

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Abstract

The effect of micro-architectural structure of cabbage (*Brassica oleracea* var. *capitata* L.) substratum and or background bacterial flora on the growth of *Listeria monocytogenes* as a function of incubation temperature was investigated. A cocktail mixture of *Pseudomonas fluorescens*, *Pantoea agglomerans* and *Lactobacillus plantarum* was constituted to a population density of approximately 5 log CFU/ml in order to pseudo-simulate background bacterial flora of fresh-cut cabbage. This mixture was co-inoculated with *L. monocytogenes* (approximately 3 log CFU/ml) on fresh-cut cabbage or in autoclaved cabbage juice followed by incubation at different temperatures (4–30 °C). Data on growth of *L. monocytogenes* were fitted to the primary growth model of Baranyi in order to generate the growth kinetic parameters of the pathogen. During storage, microbial ecology was dominated by *P. fluorescens* and *L. plantarum* at refrigeration and abuse temperature, respectively. At all temperatures investigated, lag duration (λ, h), maximum specific growth rate ($\mu_{\max}$, h^{-1}) and maximum population density (MPD, log CFU/ml) of *L. monocytogenes* were only affected by medium micro-architectural structure, except at 4 °C where it had no effect on the $\mu_{\max}$ of the pathogen. Comparison of observed values of $\mu_{\max}$ with those obtained from the Pathogen Modelling Program (PMP), showed that PMP overestimated the growth rate of *L. monocytogenes* on fresh-cut cabbage and in cabbage juice, respectively. Temperature dependency of $\mu_{\max}$ of *L. monocytogenes*, according to the models of Ratkowsky and Arrhenius, showed linearity for temperature range of 4–15 °C, discontinuities and linearity again for temperature range of 20–30 °C. The results of this experiment have shown that the constituted background bacterial flora had no effect on the growth of *L. monocytogenes* and that micro-architectural structure of the vegetable was the primary factor that limited the applicability of PMP model for predicting the growth of *L. monocytogenes* on fresh-cut cabbage. A major limitation of this study however is that nutrient profile of the autoclaved cabbage juice may be different from that of the raw juice thus compromising realistic comparison of the behaviour of *L. monocytogenes* as affected by micro-architectural structure.

Keywords: Micro-architectural structure; Cabbage substratum; Background bacterial flora; *Listeria monocytogenes*

1. Introduction

Listeria monocytogenes is a psychrotrophic food-borne pathogen capable of growing at refrigeration temperatures (Walker et al., 1990), relatively low pH (George et al., 1988) and has been isolated from raw vegetables (Beuchat, 1996; Nguyen-the and Carlin, 2000). Raw vegetables have increasingly been associated with food-borne diseases possibly as a result of modern agricultural practices, such as irrigation with polluted water or fertilization with manure, sewage, sludge and animal excreta (Beuchat, 2002). Although increased consumer interest in the consumption of fresh vegetables gives a positive premise for the growth of fresh vegetable industry, the psychrotrophic character of *L. monocytogenes* is still a major challenge since refrigeration technology alone can no longer control the growth of this pathogen.

Modelling the growth and survival of food-borne pathogenic bacteria is nowadays considered as a basic tool for the

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prediction of food safety (McMeekin et al., 1993) and has proved to be a better alternative to the traditional, time-consuming microbiological enumeration techniques. Several bacterial growth models constructed under constant or fluctuating environmental conditions have been published (Zwietering et al., 1990; Pruit and Kamau, 1993; Baranyi and Roberts, 1994; Skinner et al., 1994; Baranyi et al., 1995; Rosso et al., 1995; Riva et al., 2001). Although some predictive models have been generated in real foods (Whitting and Masana, 1994; Farber et al., 1995; Murphy et al., 1996; Ross et al., 2000), most models have been derived from experimental data generated in broth using pure cultures in varying environmental conditions (temperature, pH, a_w, salt concentration, gas atmosphere and or preservative content) (Buchanan, 1991; McMeekin et al., 1993; Adir and Briggs, 1993; Jones and Walker, 1993). The US Department of Agriculture (USDA) Service's Pathogen modelling Program (PMP), a widely used pathogen prediction model, was designed using broth-based data on pure culture of *L. monocytogenes* (Buchanan et al., 1989; Buchanan and Philips, 1990). However, for a commodity like fresh-cut cabbage, *L. monocytogenes* when present will always co-exist with spoilage microorganisms. Therefore, factors such as interspecies competition for nutrients, production of antimicrobial agents by spoilage microorganisms may interfere with and modify growth of the pathogen. The presence of *L. monocytogenes* on minimally processed vegetables is reported to be associated with significant presence of Pseudomonads, Enterobacteriacae and lactic acid bacteria (Nguyen-the and Carlin, 1994, 2000; Francis and O'Beirne, 1998; Palmai and Buchanan, 2002), which means that, in reality, *L. monocytogenes* does not grow on minimally processed vegetables as pure culture, but rather in competition with natural background microflora. Furthermore, survival and growth of microorganisms in food is not only affected by chemical composition of the food and storage environment, as always simulated in broth experiments, but also by the micro-architectural structure of the food (Wilson, 2000).

Buchanan and Bagi (1997) observed a decrease in maximum population density of *L. monocytogenes* in a mix culture with *Carnobacterium piscicola* as a function of pH, temperature and NaCl concentration in broth and the extent of growth suppression was dependent on the relative growth rate of each species. A similar study was done by Pin and Baranyi (1998) on some groups of bacteria found in meat. These authors found significant differences in growth response of those bacterial species as a function of pH and temperature when grown separately or together. Although microbial interactions were observed in these studies, data were obtained from broth experiments and, by consequence, structural architecture of relevant foods were not considered. Also other studies on interactions between *L. monocytogenes* and background microflora have mostly concentrated on co-cultures of two species (Marchall and Schmidt, 1988; Farrag and Marth, 1989; Freedman et al., 1989, 1991; Ashenafi, 1991; Jeppesen and Huss, 1993; Breidt and Fleming, 1998; Buchanan and Bagi, 1999; Campo et al., 2001). In reality, however, background microbial populations on fresh produce are very diverse (Nguyen-the and Carlin, 1994, 2000; Bennik et al., 1996). The objective of this study was therefore to investigate the effect of micro-architectural structure of cabbage substratum and or background bacterial flora consisting of a cocktail mixture of *P. fluorescens*, *P. agglomerans* and *L. plantarum* on growth of *L. monocytogenes*.

2. Materials and methods

2.1. Bacterial strains and culture preparation

Strains of *L. monocytogenes*, *Pseudomonas fluorescens*, *Lactobacillus plantarum* and *Pantoea agglomerans*, locally isolated from cabbage in Uganda were used. The cultures were maintained at −80 °C in tryptic soy broth (TSB; Oxoid, Basingstoke, UK) containing 10% glycerol (BDH, London, UK). The cultures were separately transferred into 10 ml of brain heart infusion broth (BHI: Merk, Darmstadt, Germany). *L. monocytogenes* was incubated at 37 °C, while *P. fluorescens*, *L. plantarum* and *P. agglomerans* were each incubated at 30 °C for 24 h with agitation at a rate of 150 rpm (Schimadzu, Kyoto, Japan). The 24-h cultures were transferred to new tubes of BHI and incubated for another 24 h under the same conditions previously mentioned.

2.2. Vegetable preparation and inoculation

Cabbages grown under sterile environmental conditions were obtained from the plant biotechnology unit of the National Crops Resources Research Institute in Uganda. Heads of cabbage (20) were harvested into sterile plastic bags using sterile scalpels. After discarding the wrapper leaves and removing the core, the leaves were cut into pieces of 3 × 3 cm using sterile surgical knife on a sterile working table followed by determination of water activity and pH. Water activity was determined using a portable water activity kit (Decagon Devices, Washington, USA) while pH was determined using the HI198129 pH meter (Hanna Instruments, Bedfordshire, UK). The populations of naturally occurring microorganisms on the cut vegetable pieces were reduced by washing with 200 mg/l sodium hypochlorite (BDH, UK) for 3 min and rinsed with sterile distilled water. Total viable microbial count on the washed cabbage pieces was determined by stomaching 10 g samples for 1 min in 90 ml of 0.1% peptone water (Merk) using Colworth Stomacher 400 (Steward Laboratory, London, UK) followed by plating on Nutrient Agar (Merk). The plates were aerobically incubated for 48 h at 30 °C. The washed vegetable pieces were divided into two batches. One batch consisted of 200 g of cut leaf samples in a plastic bag (30 × 40 cm). The bags were inoculated with *L. monocytogenes* alone or *L. monocytogenes* together with a cocktail mixture of *P. fluorescens*, *P. agglomerans* and *L. plantarum* at room temperature (25 °C). The inoculum sizes used were approximately 3 log CFU/g for *L. monocytogenes* and 5 log CFU/g cocktail mixture of equal concentrations of *P. fluorescens*, *P. agglomerans* and *L. plantarum*. The bags were gently shaken for 1 min to allow even distribution of bacterial cells. 10 g of inoculated cabbage pieces were weighed into 400 ml stomacher bags (The oxygen, carbon dioxide and water permeability characteristics of the plastic material were not determined). The bags were then incubated aerobically at 4, 7, 10, 15, 20, 25 and 30 °C for various days depending on temperature. The other batch of cut leaf samples was macerated in a home kitchen blender (Philips, Tokyo, Japan) and filtered through a 100-micron sieve. The filtrate was sterilized for 15 min in an autoclave at 121 °C. The sterilized juice was cooled to room temperature and dispensed in sterile plastic bags. The juice was then inoculated with the test organisms at the same ratio as in the case of fresh-cut cabbage. The bags were also stored aerobically at the same temperatures as mentioned before. Sample units were withdrawn at appropriate intervals depending on incubation temperature to determine the population of different microbial species as described below.

2.3. Enumeration of microorganisms

To the 10 g sample in stomacher bag, 90 ml of 0.1% peptone water (Merk) was added followed by homogenisation for 1 min using Colworth Stomacher 400. Ten-fold dilution series were made using 0.1% peptone water (Merk) and plated in duplicate on appropriate selective media. *L. monocytogenes* was incubated for 24 h at 37 °C on Oxford Listeria selective agar supplemented with Oxford Listeria supplement (Merk). *P. fluorescens* was plated on *Pseudomonas* agar base (Oxoid) with CFC supplement SR 103 (Oxoid), and incubated at 30 °C for 24 h. *P. agglomerans* was enumerated in Violet Red Bile Glucose Agar (Oxoid) using pour plate method with an overlayer and was incubated for 48 h at 37 °C. *L. plantarum* was enumerated using pour plate method with an overlayer followed by 3 days incubation at 30 °C on *Lactobacilli* Agar (Oxoid). For the cabbage juice experiment, the bags were shaken vigorously to ensure uniform distribution of the organisms. 5 ml of juice from each bag was appropriately diluted and plated as in the case of fresh-cut cabbage. The experiment was repeated three times and all determinations were made in duplicates.

2.4. Data analysis

Growth curves of *L. monocytogenes* (log population versus time) were fitted using the DMfit Excel Add-In software which is based on the model of Baranyi et al. (1993) and the growth parameters (λ, $\mu_{\max}$ and MPD) were generated. This software is available through the website of the UK Institute of Food Research (www.ifr.bbsrc.ac.uk). The *R* statistical software (www.r-project.org) was used for data analysis. At each growth temperature, One-way ANOVA was used to test the significance of the effects of the constituted background bacterial flora and/or micro-architectural structure of cabbage substratum on growth parameters of *L. monocytogenes*. The means were separated using Tukey's HSD test at 5% level of significance. The observed and PMP-predicted values of $\mu_{\max}$ of *L. monocytogenes* were compared mathematically using the bias and accuracy factor indices described by Ross (1996). The PMP-predicted $\mu_{\max}$ was derived from generation time (T_g) according to Eq. (1).

$$\mu_{\max} = \frac{\ln 2}{T_g}. \tag{1}$$

Finally, temperature dependency of $\mu_{\max}$ of *L. monocytogenes* was checked using the square root model of Ratkowsky et al. (1982); Eq. (2) and Arrhenius method (Labuza and Fu, 1993); Eq. (3), respectively.

$$\sqrt{\mu_{\max}} = b(T - T_{\min}) \tag{2}$$

$$\ln k = \ln k_0 - \frac{E_A}{RT}. \tag{3}$$

3. Results and discussion

3.1. Experimental design

In this study, we constituted a mixed culture of *L. plantarum*, *P. fluorescens* and *P. agglomerans* in order to simulate background bacterial flora of fresh-cut cabbage. We designed the experiment to check whether the constituted background bacterial flora and or micro-architectural structure of cabbage substratum limits the growth of *L. monocytogenes*. Micro-architectural structure refers to the structural features of the aqueous regions of the food where microbial growth takes place (Wilson et al., 2002). The initial inoculum level (3 log CFU/g) for *L. monocytogenes* used in this experiment has been applied in similar studies by other researchers (Robinson et al., 1998; Campo et al., 2001; Koseki and Isobe, 2005) and is just 1 log more than the risk level of 2 log CFU/g for people with increased susceptibility (ICMSF, 1994). The reason for the selection of the three spoilage bacteria was based on the premise that *P. fluorescens*, *P. agglomerans* and *L. plantarum* would be representative for Pseudomonads, *Enterobacteriacae* and lactic acid bacteria, respectively; the predominant groups of background bacterial flora on fresh-cut vegetables. But nevertheless, other groups of bacteria as well as yeasts and molds, are also normal components of the microflora of raw vegetables. Total plate count analysis showed that the cabbage used had no detectable microbial load at a detection limit of 2 log CFU/ml (data not shown). Cultivation of the test organisms in sterile cabbage is not a realistic reflection of commercial fresh-cut cabbage. However, sterile cabbage was used so that population dynamics of each of the inoculated bacterial species could be easily and accurately followed. It is however notable that autoclaving cabbage juice might have altered nutrient composition of the juice, thereby altering the growth rates of the test organisms. This is a subject of further research.

3.2. Evolution of microbial populations during storage

Population dynamics of *L. monocytogenes* and the background bacterial flora on fresh-cut cabbage or in cabbage juice are shown in Fig. 1A, B, C and D. Only results for storage at 4

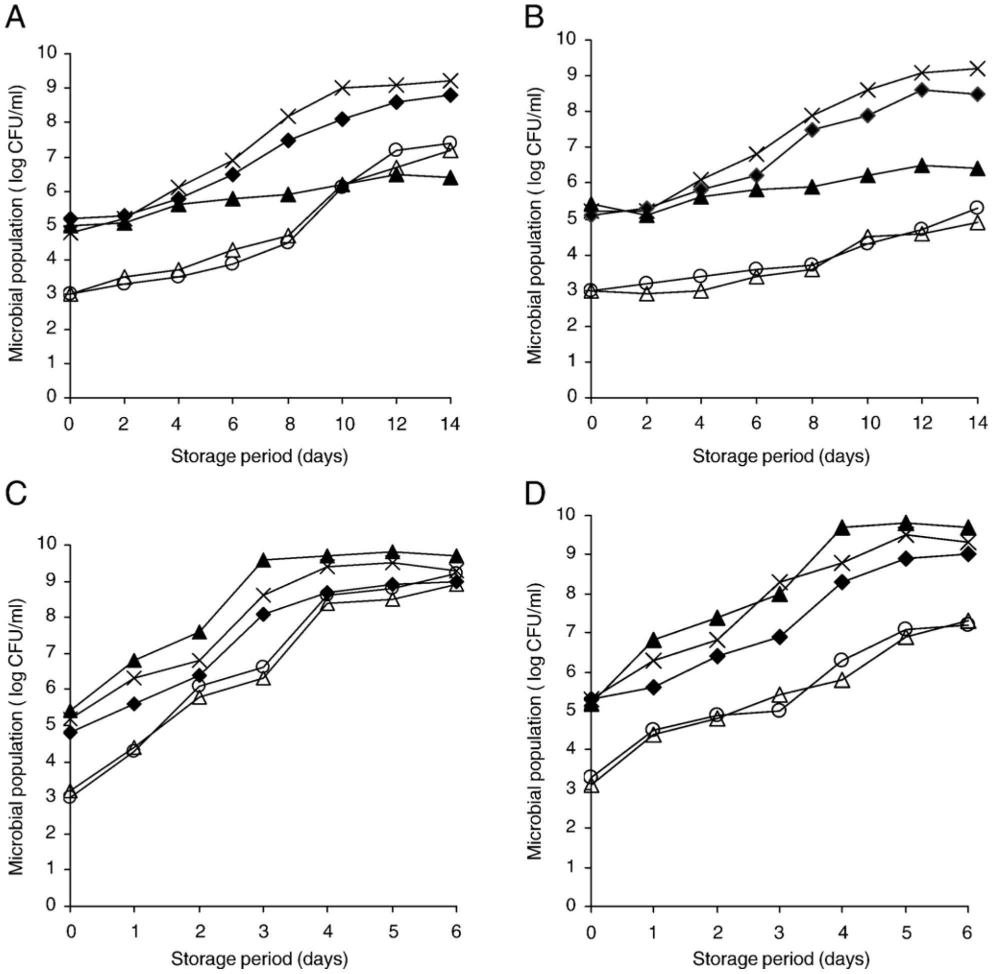

Fig. 1. Evolution of microbial population in cabbage juice during storage at 4 °C (A) or 30 °C (C) and on shredded cabbage at 4 °C (B) or 30 °C (D). (○): pure culture of *L. monocytogenes*; (△): *L. monocytogenes* in co-culture with *P. fluorescens*, *L. plantarum* and *P. agglomerans*; (◆): *P. agglomerans*; (▲): *L. plantarum*; (×): *P. fluorescens*.

and 30 °C are presented; representing normal temperature in distribution chains and temperature abuse, respectively. During storage at 4 °C (Fig. 1A and B) or 30 °C (Fig. 1C and D), all organisms grew substantially except *L. plantarum* whose growth was highly constrained at 4 °C; which could be due to the non-psychrotrophic nature of *L. plantarum*. However, growth of all the organisms was faster at 30 °C than at 4 °C; which is logical since metabolic activities of the organisms are expected to increase with temperature. More specifically, *P. fluorescens* dominated microbial ecology of both cabbage juice and fresh-cut cabbage at 4 °C (Fig. 1A and B). The low nutritional demand of *P. fluorescens* could account for its overgrowth. However, at the end of storage at 30 °C, microbial ecology shifted to the dominance of *L. plantarum* (Fig. 1C and D); which is rather strange since *L. plantarum* has high nutritional demand and is not expected to dominate microbial ecology of cabbage, unless salt is added to suppress the growth of other competing organisms e.g. the production of sauerkraut. Perhaps the absence of other bacterial genera, yeasts and molds that are naturally found on cabbage may be the factor that contributed to the unusual behaviour of *L. plantarum*. This is also a subject of further investigation.

Comparison between Fig. 1A and B or Fig. 1C and D shows that growth of all the organisms (except *L. plantarum* at 4 °C) was faster in cabbage juice than on fresh-cut cabbage; which could be attributed to differences in micro-architectural structures of cabbage substratum. Furthermore, Fig. 1A, B, C and D show that at 4 and at 30 °C, *L. monocytogenes* attained much higher populations in cabbage juice than on fresh-cut cabbage irrespective of the presence of the constituted background bacterial flora. This implies that accumulation of *L. monocytogenes* during storage was only constrained by micro-architectural structure of the vegetable. Conner et al. (1986) reported that two strains of *L. monocytogenes* (Scott A and LCDC 81-861) grew from 4 to 9 log CFU/ml within 8 days in autoclaved un-clarified cabbage juice when stored at 30 °C, but that the organisms did not grow when the juice was stored at 5 °C for 70 days. The non-psychrotrophic character of the two strains (Scott A and LCDC 81-1861) in cabbage juice as observed by these authors is in contrast with our findings. However, growth of *L. monocytogenes* strains (Scott A and LCDC 81-861) on other cruciferous vegetables at 4 °C was later observed by other authors (Berrang et al., 1989), showing that the two strains were indeed psychrotrophic. Malakar et al.

(2003), using data on inhibition of *L. monocytogenes* in broth, showed that pathogen-background microflora interactions were only important at high population densities (~8 log CFU/g); which is irrelevant in the case of fresh-cut vegetables since spoilage would already occur. With regard to substrate micro-architectural structure, reports available in literature show that substrates with "surface" micro-architecture (e.g. vegetables) affect microbial growth indirectly by inducing constraints on mechanical distribution of water (Hills et al., 1996, 1997), chemical redistribution of organic acids and preservatives (Brocklehurst et al., 1993) and physical constraint on mobility of microorganisms (Mattila and Frost, 1988a,b; Dodd, 1990; Dood and Waites, 1991; Robins et al., 1994; Robins and Wilson, 1994; Wimpenny et al., 1995).

Assuming that commercial fresh-cut cabbage has initial microbial load similar to the level used in this experiment, then accumulation of *L. monocytogenes* on the vegetable will normally not be hindered by background microflora. Aytac and Gorris (1994) reported that *L. monocytogenes* proliferated very well on chicory which had relatively low initial microbial load. Similar results were also reported for endive (Carlin et al., 1996) and shredded lettuce (Francis and O'Beirne, 1997). Our results also agree fairly well with the findings of Francis and O'Beirne (1998) which show that inoculum size of indigenous microflora of lettuce similar to the one used in this experiment, had no effect on the survival or growth of *L. innocua*.

3.3. *Primary modelling of growth parameters of L. monocytogenes*

Fig. 2A shows plots of λ of *L. monocytogenes* as function of temperature. Generally, λ of *L. monocytogenes* increased with decreasing incubation temperature, irrespective of the effects of medium micro-architectural structure and/or background bacterial flora. λ of *L. monocytogenes* in cabbage juice was significantly shorter than observed on fresh-cut cabbage at all temperatures, irrespective of the presence of the constituted background bacterial flora ($P \leq 0.05$). However, there was no significant difference in λ of *L. monocytogenes* between the monoculture experiments and mixed culture experiments in cabbage juice or on fresh-cut cabbage ($P > 0.05$). A critical look at Fig. 2A reveals that both micro-architectural structure and temperature affected λ of *L. monocytogenes* between 4 and 15 °C. However, beyond 15 °C λ became independent of storage temperature and was only affected by differences in substrate micro-architecture. The fact that λ of *L. monocytogenes* was only temperature dependent between 4 and 15 °C suggests that pre-incubation temperature (37 °C) affected λ of

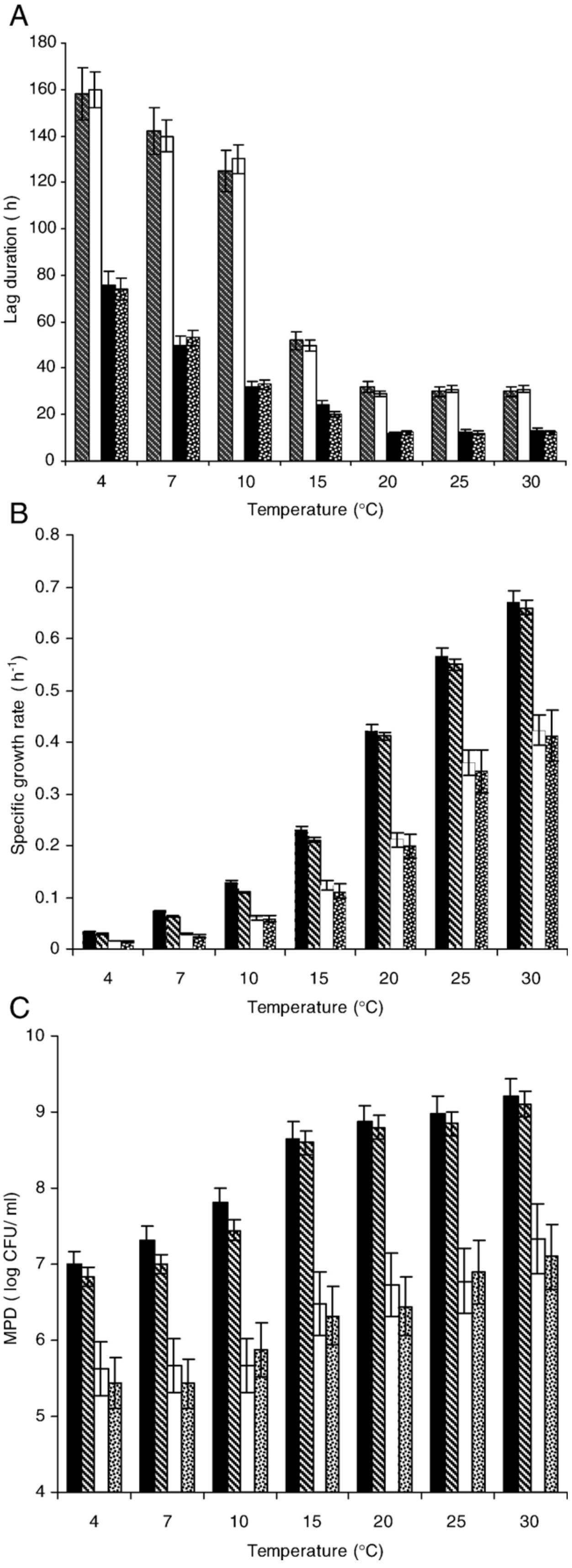

Fig. 2. Growth parameters of *L. monocytogenes* in cabbage juice or on shredded cabbage as pure culture or in co-culture with *P. fluorescens*, *P. agglomerans* and *L. plantarum* at different temperatures. (■): Pure culture of *L. monocytogenes* in cabbage juice; (▧): *L. monocytogenes* in co-culture with *P. fluorescens*, *P. agglomerans* and *L. plantarum*; (□): Pure culture of *L. monocytogenes* in shredded cabbage; (░): *L. monocytogenes* in co-culture with *P. fluorescens, P. agglomerans* and *L. plantarum*. A: Lag phase duration (λ, h), B: Specific growth rate ($\mu_{\max}$, h^{-1}) and C: Maximum population density (MPD, log CFU/ml).

the pathogen when cultured at temperature range of 4–15 °C; which is probably due to the effect of temperature downshift (Francois et al., 2007). Bernaerts et al. (2002) studied the effect of temperature shift on the growth of *E. coli*-K12 and observed that a large temperature shift from 17.5 °C to 32.5 °C resulted in an intermediate λ, while a relatively small temperature change from 22.5 °C to 27.5 °C resulted in immediate adjustment to the new growth conditions. Hudson (1993) examined the influence of four prior incubation temperatures on λ of *Aeromonas hydrophila* and observed that minimum transitions occurred when the pre-incubation temperature matched the actual incubation temperature, longer lag phases appeared when cells from high pre-incubation temperatures were transferred to lower temperatures.

Fig. 2B shows plots of $\mu_{\max}$ of *L. monocytogenes* against temperature. For all temperatures (except 4 °C), $\mu_{\max}$ of *L. monocytogenes* in cabbage juice was significantly greater than observed on fresh-cut cabbage, irrespective of the presence of the constituted background bacterial flora ($P \leq 0.05$). However, there was no significant difference in $\mu_{\max}$ of the pathogen between monoculture experiments and mixed culture experiments in cabbage juice or on fresh-cut cabbage ($P > 0.05$); suggesting that $\mu_{\max}$ of *L. monocytogenes* was only affected by differences in substrate micro-architecture. Robinson et al. (1998) defined fitness of a medium to support growth of microorganisms in terms of $\mu_{\max}$ and suggested that differences between media can be quantified in terms of differences in $\mu_{\max}$ of a particular organism. In this experiment, fresh-cut cabbage represented surface micro-architecture while cabbage juice simulated liquid micro-architecture, respectively. Literature shows that microbial growth in liquid medium is typically planktonic and faster than growth in the case of surface micro-architecture where microbial cells are immobilised and constrained to grow as colonies (Wilson et al., 2002). Microbial colonies on surfaces are reported to be highly affected by diffusion limitations, oxygen depletion and proton accumulation (Peters et al., 1987; Robinson et al., 1991). Brocklehurst et al. (1997) compared growth rates of surface colonies of *Salmonella typhimurium* with cells growing planktonically in liquid medium and found that growth in liquid was faster than on the surface; which is consistent with our results.

With regard to MPD, Fig. 2C shows that at all storage temperatures investigated, MPD of *L. monocytogenes* on fresh-cut cabbage was significantly lower than in cabbage juice, irrespective of the presence of the constituted background bacterial flora ($P \leq 0.05$). There was a difference of about 1.5–2 log CFU/ml. However, there was no significant difference in MPD of the pathogen between monoculture experiments and mixed culture experiments in cabbage juice or on fresh-cut cabbage ($P > 0.05$). These observations also suggest that micro-architectural structure of fresh-cut cabbage was the primary factor that limited the ability of *L. monocytogenes* to multiply and to reach the highest achievable population while the constituted background bacterial flora had no effect. These results are consistent with the findings of Koseki and Isobe (2005) which show that the MPD of *L. monocytogenes* inoculated at a rate of 3.5 log CFU/g on lettuce was not affected by initial background microbial load approximated at 5.6 log CFU/g.

3.4. Temperature dependency of μ_{max} of *L. monocytogenes*

Temperature dependencies of $\mu_{\max}$ of *L. monocytogenes* according to Ratkowsky et al. (1982) exhibited the following scenarios: linearity between 4 and 15 °C, discontinuities, and linearity again between 20 and 30 °C (Fig. 3A). Robinson et al. (1998) got fairly similar results with *L. monocytogenes* in model media where the relationship was linear within temperature range of 5–25 °C; beyond discontinuities were also observed. Our results agree fairly well with those of Koseki and Isobe

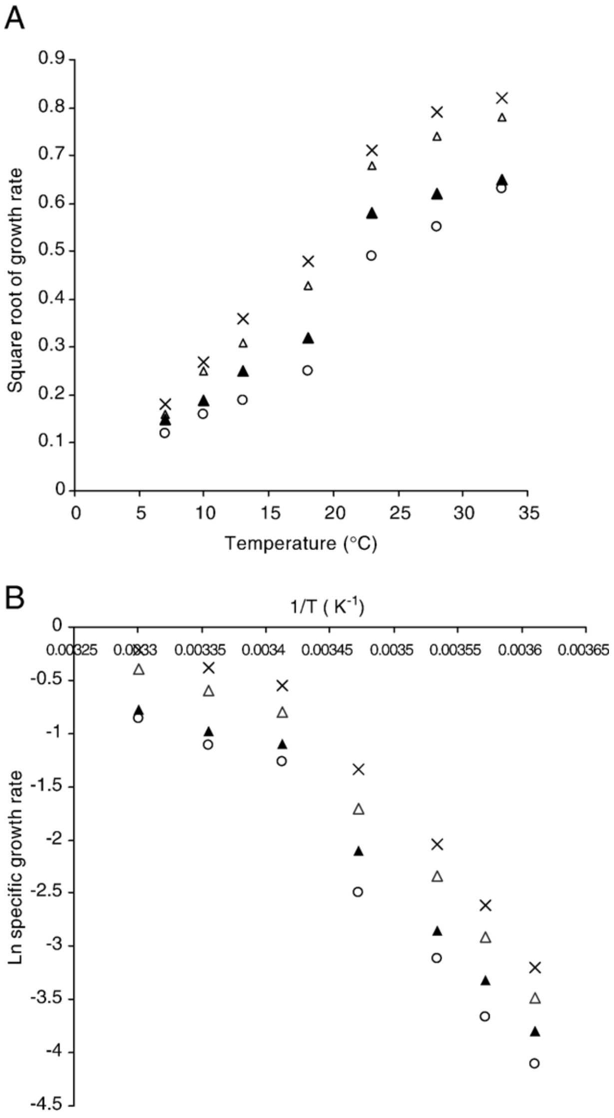

Fig. 3. Secondary models for growth rate of *L. monocytogenes* in cabbage juice or on shredded cabbage as pure culture or in co-culture with *P. fluorescens*, *P. agglomerans* and *L. plantarum*. (×): Pure culture of *L. monocytogenes* in cabbage juice; (▲): *L. monocytogenes* in co-culture with P. *fluorescens*, *P. agglomerans* and *L. plantarum*; (△): Pure culture of *L. monocytogenes* on shredded cabbage; (○): *L. monocytogenes* in co-culture with *P. fluorescens*, *P. agglomerans* and *L. plantarum*. A: Square root model (Ratkowsky) and B: Arrhenius model.

(2005) where, for *L. monocytogenes*, linear relationships were observed on fresh-cut lettuce for temperature range of 5–25 °C. The dependency of $\mu_{\max}$ on temperature for *L. monocytogenes* according to Arrhenius model also exhibited linearity for temperature range of 4–15 °C, discontinuities and linearity again between 20 and 30 °C (Fig. 3B). The results of Robinson et al. (1998) for *L. monocytogenes* show non-linear relationship of the Arrhenius model between 5 and 37 °C, a conclusion that could be attributed to too wide temperature range. It should be noted that the Arrhenius equation was derived empirically based on thermodynamic considerations (Labuza and Riboh, 1982). However, bacterial growth in foods is a complex phenomenon, which makes the Arrhenius relationship inadequate for fitting data well below optimal or above minimal temperature for growth (McDonald and Sun, 1999). Data available in literature show that Arrhenius plots are normally only accurate over a limited temperature range for microbial growth (Labuza and Fu, 1993) and is well illustrated with data on the growth rate of *Pseudomonas fragi* (Fu et al., 1991). Other authors have found the equation totally inadequate for secondary modelling of temperature effects in food systems (Ratkowsky et al., 1982; Standard et al., 1985; Phillips and Griffiths, 1987). From our observations it can be concluded that both the Arrhenius and Ratkowsky models can only be used to describe $\mu_{\max}$ data for temperature range of 4–15 °C or 20–30 °C but not the entire span of the temperature range investigated.

3.5. Discrepancies between PMP-predicted and observed μ_{max}

The PMP-predicted data on $\mu_{\max}$ of *L. monocytogenes* were generated under a pH of 5.8 and water activity of 0.98 for cabbage (experimentally determined). Comparison of observed values of $\mu_{\max}$ for *L. monocytogenes* with the PMP-predicted values using the bias and accuracy factor indices (Ross, 1996) revealed that PMP overestimated the growth rate of *L. monocytogenes* on fresh-cut cabbage and in cabbage juice, respectively. The $\mu_{\max}$-bias and $\mu_{\max}$-accuracy factor of the PMP prediction was on average 3.6 and 1.82 on fresh-cut cabbage, respectively. On the other hand, $\mu_{\max}$-bias and $\mu_{\max}$-accuracy factor of the PMP prediction for cabbage juice were on average 1.3 and 0.3, respectively. The discrepancies between PMP-predicted values of $\mu_{\max}$ and those observed on fresh-cut cabbage and in cabbage juice could be attributed to differences in growth environment (Koseki and Isobe, 2005) and poor nutrient content of cabbage compared to the nutritious broth medium from which PMP model was developed (Buchanan et al., 1989; Buchanan and Philips, 1990). The difference in accuracy or bias factor of the PMP prediction between fresh-cut cabbage and cabbage juice could also be caused by differences in micro-architectural structure of cabbage substratum. Our findings with *L. monocytogenes* on fresh-cut cabbage are quite consistent with observations of other researchers on various foods (Dalgaard and Jorgensen, 1998; Meldrum et al., 2003; Koseki and Isobe, 2005). Such a conservative “fail-safe” phenomenon of predictive models as experienced with the PMP model in this experiment is well discussed by other authors (Ross et al., 2000; Wilson et al., 2002).

4. Conclusions

The results of this experiment have given substantial evidence that pathogen-background bacterial flora interactions at population densities commonly found on fresh-cut vegetables is irrelevant and that micro-architectural structure of the vegetable is the principal factor responsible for the conservative, “fail-safe” phenomenon of the PMP model when used to predict the growth of *L. monocytogenes* on fresh-cut vegetables.

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