Food Microbiology, 2001, **18**, 95–102 doi:10.1006/fmic.2000.0380
Available online at http://www.idealibrary.com on IDEAL®

ORIGINAL ARTICLE

Survival of hepatitis A virus on modified atmosphere-packaged (MAP) lettuce

S. Bidawid[1]*, J. M. Farber[1], S. A. Sattar[2]

Experiments were designed to study the effect of various modified atmospheres (MA) on the survival rate of hepatitis A virus (HAV) on lettuce. Pieces of lettuce inoculated with HAV were incubated at room temperature (RT) and 4°C for 12 days in ambient air and under various modified atmospheres (CO_2:N_2 at 30:70, 50:50, 70:30 and 100% CO_2) inside plastic bags of low O_2 permeability. Samples were removed on days 1, 3, 6, 9 and 12 and the virus was recovered and plaque-assayed to determine residual titer. Incubation for 12 days at 4°C showed that the lowest HAV survival rate (47·5%) was on lettuce stored in a petri-dish (atmospheric air), whereas the greatest survival rates (83·6%) was on lettuce stored under 70% CO_2. Statistical analysis of virus survival at 4°C indicated that HAV titers decreased for all packages, but without a significant ($P > 0·05$) difference between the package types. At RT, however, a significantly ($P < 0·05$) lower HAV survival rate (0·01%) was evident on lettuce stored in a petri dish, whereas survival rates as high as 42·8% were observed on lettuce stored under 70% CO_2; much lower survival rates ($\leqslant 8·6\%$) were obtained on lettuce stored under other MAP environments at RT. Statistical analysis of the RT data indicated that there was a highly significant ($P < 0·05$) decrease in HAV titre with increasing storage time and between package types, except for lettuce stored under 70% CO_2. These data indicate that MAP does not influence HAV survival when present on the surface of produce incubated at 4°C. A slight improvement in virus survival on lettuce was seen in the presence of high CO_2 levels at RT. This may have been attributed to the inhibition of spoilage-causing enzymatic activities in the lettuce, which may have reduced exposure of the virus to potential toxic by-products.

Introduction

Modified atmosphere packaging (MAP) is a technique commonly used by the food industry to prolong the shelf-life of foods. Almost 15% of California iceberg lettuce (Brody 1995), 75% of California strawberries, 4% of all fresh vegetables in the USA, and up to US$40 million worth of fresh-cut vegetables for retail are distributed under MAP conditions. Overall, MAP technology represents up to US$4 billion in sales of fresh produce (Anon. 1991), and is expected to increase in the future.

MAP involves the packaging of food under an atmosphere which is different from the normal air composition (78·08% N_2, 20·96% O_2, 0·03% CO_2, variable traces of water and of inert

*Corresponding author. Health Canada Food Directorate, Bureau of Microbial hazards Sir F. G. Banting research Centre, Ross Ave., Tunney's Pasture Postal locator #: 2204A2 Ottawa, Ontario, Canada, K1A 0L2. Fax:(613) 941-0280.
E-mail: Sabah_Bidawid@hc-sc.gc.ca

Received: 5 July 2000

[1]Health Canada, Food Directorate, Bureau of Microbial Hazards, Ottawa, Ontario, Canada.
[2]Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ontario, Canada

0740-0020/01/010095 +08 $35.00/0

gases). Three gases are generally used in MAP: nitrogen (N_2), oxygen (O_2), carbon dioxide (CO_2) or various mixtures of the three. The choice of gas mixture depends on the type of product and its expected shelf-life (Goodburn and Halligan 1988). An elevated CO_2 and/or reduced O_2 environment extends the shelf-life of foods by inhibiting chemical, enzymatic and microbial spoilage. This environment can selectively inhibit the growth of Gram-negative bacteria such as *Pseudomonas* spp., which, under aerobic conditions, typically grow relatively rapidly and produce the off-flavours and odours associated with the spoilage of many foods (Enfors and Molin 1981). Other bacteria, such as the lactic acid bacteria, are not usually affected by high CO_2 levels. Thus, they tend to predominate in MAP foods and help to prolong shelf life by producing compounds which inhibit spoilage bacteria (Dodds 1995).

One of the major concerns associated with the use of MAP is that of product safety. The desired suppression of spoilage micro-organisms may create opportunities for slower growing and potentially pathogenic bacteria (Genigeorgis 1985, Hintlian and Hotchkiss 1987, Farber 1991), which may render the product potentially hazardous before it is overtly spoiled (Dodds 1995). Fresh, whole produce has not traditionally been associated with foodborne disease. However, produce can become contaminated by a variety of pathogenic micro-organisms either during processing, handling by an infected foodhandler, or when irrigated with and/or harvested from fecally-contaminated soils (Cliver 1997, Jaykus 2000).

Although MAP can inhibit the growth of bacterial and fungal spoilage micro-organisms, its effect on the survival of enteric viruses, including hepatitis A virus (HAV), has not been investigated and remains unknown. Increase in demand for fresh-cut produce is associated with an increase in trade, mass production and food handling. This provides a greater potential for the contamination of foods with HAV, which is known to cause foodborne illness. Lettuce is one of the many types of vegetable foods implicated as a vehicle in HAV transmission, and contributing to hepatitis A outbreaks. Konowalchuk and Speirs (1975) demonstrated that a number of enteric viruses survived well for many days on lettuce, which they described as best suited for virus survival due to its leafy and relatively moist surface.

MAP plays an important role in suppressing the growth of spoilage bacterial and fungal micro-organisms in foods, and thus in prolonging the shelf-life of various products. Although many studies have examined the survival of both spoilage and pathogenic bacteria in various MAP environments, studies have not been conducted to investigate the effect of MAP on the survival of foodborne viruses. Since HAV is one of the common viral food contaminants, and lettuce has been implicated as a vehicle of virus transmission (Cliver 1985), experiments were designed to investigate the survival of HAV on lettuce stored at 4°C and room temperature under normal air and different MAP conditions.

Materials and Methods

Cells and viruses

Seed cultures of FRhK-4 cells and hepatitis A virus (HM-175) were kindly provided by M.D. Sobsey of the University of North Carolina, Chapel Hill, North Carolina, USA. The methods used for cultivation and maintenance of the cells and preparation of virus pools were as described previously (Mbithi et al. 1991, 1992). Virus pools were stored in 1 ml portions at −80°C.

Plaque assay

Measurements of HAV titer were done by plaque assays (Mbithi et al. 1991). Briefly, cell monolayers were grown overnight in 12-well culture plates (Costar/Fisher Scientific, Ottawa, Ontario) at 37°C, 5% CO_2 . A 100 µl portion of each virus dilution was inoculated into each of three wells. The virus was allowed to adsorb to the cells for 90 min at 37°C, and then 2 ml of a semisolid agarose-containing overlay was added to each well. Plates were incubated in a humid atmosphere at 37°C, 5% CO_2 for 8 days. The procedure used to fix and stain the mono-

layers for plaque counting has been described previously (Sattar et al. 1989).

Inoculation of lettuce with HAV

Romaine lettuce, purchased locally, was selected as a representative of a vegetable in this study. Individual lettuce leaves were cut into rectangular pieces of approximately 6×7 cm, washed with a non-germicidal liquid soap (Ivory liquid soap^{TM}, Procter and Gamble, Toronto, Ontario, Canada), thoroughly rinsed in water for 2 min and allowed to dry for approximately 20 min in a laminar flow hood. Each side (front and back) of the lettuce pieces was exposed to UV light (930 Watts) for 1 min to reduce and/or eliminate contaminating microorganisms that might have interfered with the plaque assay. Each piece of lettuce was placed in a clean and UV-disinfected polystyrene weighing boat.

To establish a baseline for virus recovery from lettuce, 10 µl of HAV (c. $1{\cdot}7 \times 10^5$ pfu; plaque-forming unit) was spread evenly over a demarcated area of approximately 2×1 cm along the length of the lettuce midrib, and then allowed to dry in a laminar flow hood for 20–30 min.

Virus recovery from inoculated lettuce

The inoculated virus on the lettuce was washed off by repeated (>25 times) pipetting of the demarcated area with 1 ml of phosphate-buffered saline (PBS), pH 7·6, through the fine end of a sterile 1-ml-capacity tip fitted onto a 1000P Gilson pipettor (Mandel, Toronto, Ontario, Canada). The virus-containing wash solution was collected from the boat, using the same tip, serially diluted, and its titer determined by the plaque assay. The percent (%) recovery rate was determined as follows:

$$\frac{\text{Titer of recovered virus}}{\text{Titer of virus at time zero}} \times 100.$$

Inoculation, incubation and processing of lettuce samples

Inoculated and uninoculated lettuce samples (in triplicates) were divided into two sets, with one being incubated at 4°C and the second at room temperature. The experimental design of this study was as follows (Fig. 1):

a) Boats containing HAV-inoculated and uninoculated lettuce pieces were placed inside 150×15 mm plastic petri dishes. Approximately 5-mm V-shape cuts were made at the corners of each boat to allow for air and humidity exchange. Moisture was provided by placing a moistened piece of cloth inside the petri dish. These experiments were designed to determine the survival of HAV on lettuce stored in open air.
b) Another batch of boats containing inoculated and uninoculated lettuce pieces were placed inside barrier plastic bags (Cryovac, 10×21 cm) of low O_2 permeability (0·46–0·93 $cm^3\,100\,cm^{-3}$ -day-atm, 4·4°C and 0% RH). The boats were packaged under the following conditions:

i) Air-packaged lettuce: bags were heat-sealed using an impulse MP-12 sealer (Chiswick, Missisauga, Ontario, Canada).
ii) MAP-packaged lettuce: bags were simultaneously flushed with specified gas mixtures and heat-sealed in a Multivac heat-seal packaging machine, model A 300/16 Type M (Knud Simonsen Industries Ltd., Rexdale, Ontario, Canada) connected to a proportional gas mixer. The gas mixtures delivered to the plastic bags consisted of the following percentages of CO_2:N_2 at 30:70, 50:50, 70:30; as well as 100% CO_2.

Samples (in triplicate) were removed on days 1, 3, 6, 9 and 12 to determine virus survival by the plaque assay. Prior to opening the plastic bags to recover the virus from the lettuce, the air inside the bags was analysed for its gas content by gas chromatography as follows: a 1-cm diameter septum (MOCON-Modern Control Inc., Minneapolis, Minnesota, USA) was firmly pressed onto each plastic bag. The needle of a Pressure-Lok® Series A-2 syringe (Chromatographic Specialty, Bockville, Ontario, Canada) was inserted through the septum, and a 0·1 ml air sample was withdrawn and was analysed with a Varian 3300 Gas Chromatography (Varian Canada Inc., Mississauga, Ontario, Canada). The results of the analysis were recorded

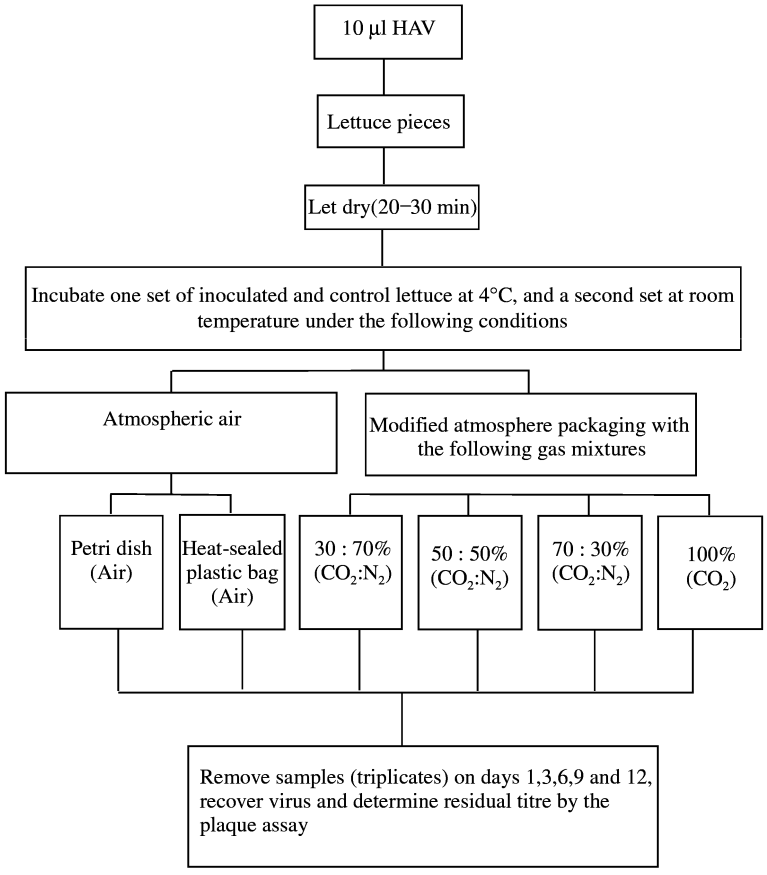

Figure 1. Schematic illustration of the study design to investigate the survival of hepatitis A virus on lettuce stored under normal and modified atmosphere environments.

and plotted automatically by a Varian 4270 Integrator (Varian Canada). The plastic bags were then cut open inside a laminar flow hood, and the boats containing the lettuce were removed. The lettuce was inspected visually to assess any changes in the color and texture. The virus was recovered from all pieces of lettuce, including those that were incubated in petri dishes.

Statistical analysis

The ANOVA model in the S-PLUS software (StatSci, MathSoft, Inc., Seattle, Washington, USA) was used to analyse the data as follows:

$$\log_{10}\text{HAV titer}_{ij} = \mu + P_i + \beta d + \gamma_i d + \varepsilon_{ij},$$

where μ is the common intercept, P_i is the intercept for package type i when adjusted for the common intercept (i.e., $P_i+\mu$), β is the overall slope, d is days of incubation, γ_i is the slope for package type i adjusted for the common slope, and ε_{ij} is the error for sample j in package type i (Miller 1966, Searle 1980). For the $_{ij}^{\text{th}}$ sample, the $\log_{10}$ HAV titer is the mean of a triplicate test sample.

Results

The efficacy of virus recovery from lettuce averaged 78·2±17·2% of the virus input. This was considered as the baseline level to determine virus survival on lettuce under various conditions. Visual inspection of the lettuce incubated at 4°C indicated that on day 6, some pieces of lettuce showed minimal browning,

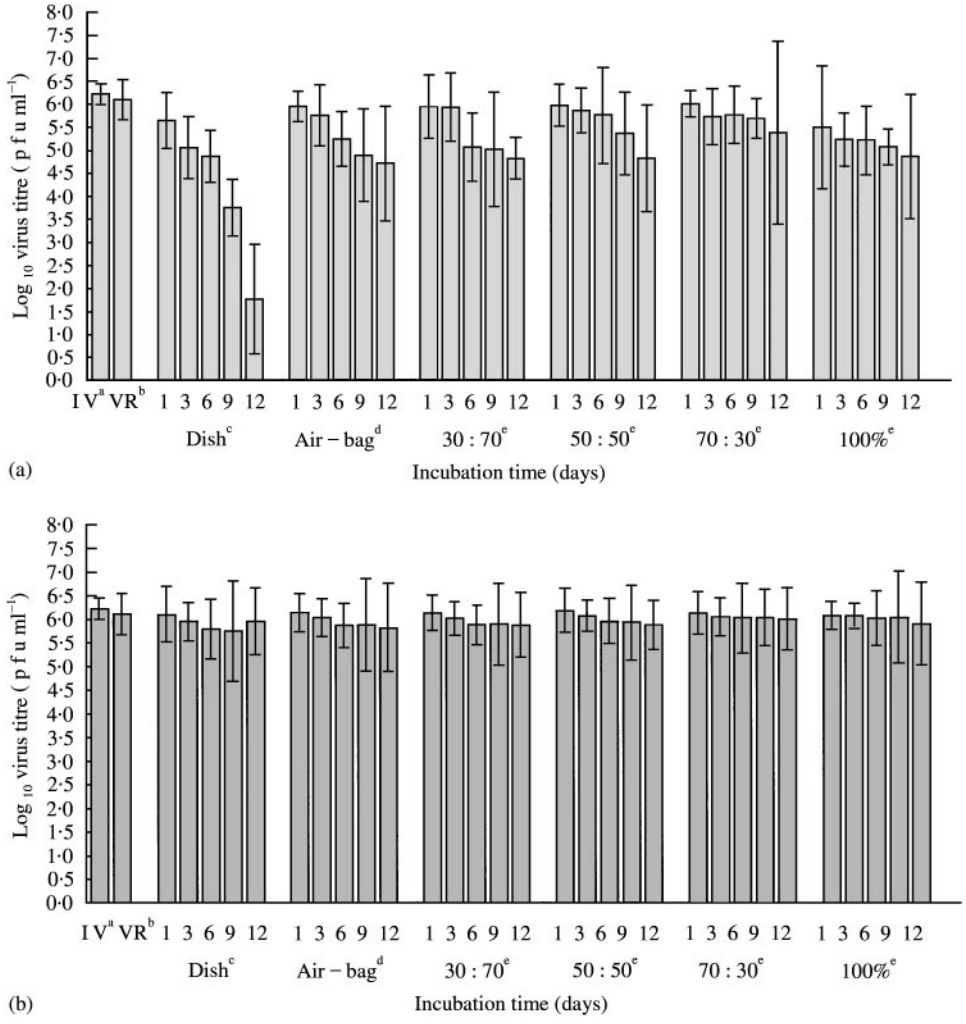

Figure 2. Survival of hepatitis A virus on lettuce stored under air and various modified atmospheres at (a) room temperature and (b) 4°C. Inoculated and uninoculated lettuce pieces were incubated for 12 days. Samples were removed on days 1, 3, 6, 9 and 12 and the titer of the residual virus was determined by the plaque assay.

[a]Input virus titer at time zero.

[b]10 µl of HAV deposited onto lettuce, allowed to dry and then recovered. The percentage recovery rate was determined (Materials and Methods), and was considered as the virus recovery baseline.

[c]Inoculated and uninoculated lettuce pieces were placed inside a loosely-covered petri dish to allow for air flow to the lettuce.

[d]Inoculated lettuce sealed inside plastic bags with normal atmospheric air.

[e]Inoculated lettuce sealed inside plastic bags with CO_2:N_2 atmospheres of 30:70, 50:50, 70:30 or 100% CO_2.

mainly in those incubated in the petri dish and in bags sealed in ambient air. Browning was also observed in pieces of lettuce incubated under 100% CO_2. For lettuce incubated at room temperature, a similar but more prominent pattern of browning was observed. This pattern continued until day 12, by which time more than 50% of the area of the lettuce leaves in the petri dish were brown. Gas chromatography analysis indicated that in most sealed bags, including heat-sealed bags containing ambient air, the ratio of gas mixtures initially flushed in

Table 1. Survival of hepatitis A virus on lettuce after storage for 12 days at 4°C and room temperature under normal and modified atmosphere packaging (MAP) environments[a,b]

Atmosphere	Day[c]	% Survival 4°C	*P*	% Survival room temperature	*P*
Dish[d]	12	47·5 (±15·2)[e]	>0·05	0·01 (±0·0)	<0·05
Air[f]	12	61·7 (27·2)	>0·05	6·2 (±4·1)	<0·05
30:70[g] (CO_2:N_2)	12	62·9 (±21·7)	>0·05	5·3 (±1·3)	<0·05
50:50[g] (CO_2:N_2)	12	59·8 (±15·7)	>0·05	6·9 (±3·0)	<0·05
70:30[g] (CO_2:N_2)	12	83·6 (±23·6)	>0·05	42·8 (±23·3)	>0·05
100% CO_2[g]	12	71·6 (±24·4)	>0·05	8·60 (±3·8)	<0·05

[a] The amount of HAV deposited on to lettuce at time zero = $1{\cdot}7 \times 10^5$ pfu/10 µl.
[b] Percent (%) virus recovered from the deposited inoculum at time zero = 78·2±17·2 (see formula in Materials and Methods). This was considered as the baseline (100%) for virus recovery.
[c] Lettuce samples were incubated at 4°C and room temperature for 12 days. Residual virus titer was determined on day 1, 3, 6, 9 and 12 by the plaque assay.
[d] Samples inside a petri dish which was loosely covered to allow for air-flow to the lettuce.
[e] Percent (%) virus survival (±standard error).
[f] Inoculated lettuce was heat-sealed inside a plastic bag containing normal atmospheric air.
[g] Inoculated lettuce was heat-sealed inside plastic bags flushed with various gas mixtures (CO_2:N_2; 30:70, 50:50, 70:30 and 100% CO_2).

the bags remained the same during storage. Only a marginal increase in CO_2 levels (<10%) was observed in some of the bags (data not shown).

HAV survival over the 12-day incubation period at 4°C and room temperature is illustrated in Fig. 2 (a) and (b), and the mean HAV survival rates obtained from all determinations carried out after 12 days of incubation are listed in Table 1. Storing HAV-inoculated lettuce at 4°C under various conditions, i.e., petri dish, air and various elevated CO_2 concentrations (MAP) resulted in HAV survival rates ranging between 47·5 and 83·6%, with an overall average of 64·5±21·3% (Table 1). Virus survival on lettuce stored under the various MAP conditions after 12 days of incubation at 4°C averaged 69·5±21·35%. The lowest HAV survival rate of 47·5% was seen on lettuce stored in a petri dish, whereas the highest survival rate was under 70% CO_2. Incubation under the same conditions, but at room temperature, showed HAV survival rates ranging between 0·01 and 42·8%, with an overall average of 11·6±5·9%. The lowest HAV survival rate (0·01%) was seen on lettuce stored in a petri dish, whereas the highest (42·8%) was under 70% CO_2. Virus survival on lettuce stored under the various MAP conditions after 12 days of incubation at RT averaged 15·9±7·8%. Percent and mean HAV survival rates at each of the incubation days (1, 3, 6, 9 and 12) can be seen in Table 1.

Discussion

The earlier appearance and more prominent browning of lettuce incubated at room temperature as compared to 4°C is attributed to the combined effect of elevated CO_2 levels and the incubation temperature. Zagory and Kader (1988) have shown that different foods have different levels of tolerance to CO_2 and that exposure to CO_2 levels above the tolerance limit can cause physiological damage to produce. Although it has been suggested that lettuce has a tolerance level to ⩽15% CO_2, higher concentrations (⩾30% CO_2) were used in this study to evaluate the effect of increased levels of CO_2 on virus survival on lettuce. Therefore, the higher CO_2 level caused injury to the lettuce and resulted in increased browning, particularly under 100% CO_2, at both temperatures. Overall, lettuce quality appeared to be much better when stored under MAP conditions, than when incubated in air.

Despite the fact that lettuce is a living tissue that continues to respire by consuming O_2 and releasing CO_2, there was very little change in the overall gaseous composition (<10% increase in CO_2) inside the bags, as was shown

by gas chromatography. This may be attributed to a number of factors such as the small surface area of the lettuce pieces used in this study relative to the bag, the lack of O_2, the presence of high CO_2 content in the gas mixtures which might have resulted in death of some of the lettuce tissue, as well as the low permeability of the plastic bag, which essentially restricted the movement of gases. Although lettuce is usually stored in plastic films of greater permeability in commercial settings, the use of low permeability bags was necessary to maintain the gas ratios as constant as possible to allow for more accurate evaluation of the effect of different gas concentrations on HAV survival. The effect of MAP on HAV survival on lettuce stored under different types of films used commercially, as well as the use of whole intact lettuce leaves, could be addressed in future investigation.

Statistical analysis (ANOVA) of the data obtained from lettuce incubated at 4°C showed that although the lowest rate of virus survival (47·5%) was seen on lettuce stored in petri dishes, HAV titres decreased for all packages, but without a significant difference ($P > 0{\cdot}05$) between the package types (Table 1). This finding suggests that MAP did not enhance HAV survival when present on the surface of the produce incubated at 4°C. At room temperature, however, a significant ($P < 0{\cdot}05$) decrease in HAV titer was shown with increasing storage time and between package types, except for lettuce stored under 70% CO_2 ($P > 0{\cdot}05$). The observed improvement in virus survival in the presence of high levels of CO_2 (70%) may be attributed to the inhibitory effect of CO_2 on the enzymes present in lettuce. It is known that elevated CO_2 and/or reduced O_2 can extend the shelf-life of foods by inhibiting chemical, enzymatic and microbial spoilage by either or both of the following mechanisms; (1) decreasing the production of ethylene gas, which induces rapid ripening in fruits, and premature yellowing in vegetables; (2) decreasing ethylene production reduces the chlorophyllase enzyme, thus reducing the breakdown of chlorophyll and subsequently delaying food degradation (Weichmann 1986, Martens and Baardseth 1987, Zagory 1995). Thus, elevated CO_2 levels may have reduced exposure of the virus to potential toxic by-products such as phenolic, ethanol and acetylaldehyde compounds, and thus improved its chances of survival. Furthermore, CO_2 inhibition of any epiphytic microflora that might be present, might also improve virus survival as it becomes less exposed to potentially harmful bacterial by-products.

Overall, virus survival rates were significantly ($P < 0{\cdot}001$) lower under all conditions at room temperature in comparison to survival at 4°C. Unlike bacteria, viruses are inert particles that are dependent on the metabolic activities of the host cell for their replication (Knipe 1996). Since HAV was inoculated onto the lettuce surface, the virus would be in an inert state which might not be affected by high CO_2, even if CO_2 presumably entered the virus particle. In comparison, CO_2 dissolved in the food matrix environment of the bacteria can result in a decrease in the intracellular or intercellular pH, which can slow down or inhibit the activity of many intracellular enzymes thereby affecting crucial metabolic activities (Enfors and Molin 1981, Lefevre 1991, Hanlin et al. 1995). Since CO_2 inhibits bacterial growth by lowering the pH, it might be possible that CO_2 could have an inhibitory effect on virus replication when the virus is inside a host cell. These aspects, however, require further investigation.

In conclusion, this study suggests that only at room temperature was there significantly better HAV survival in a high CO_2 environment (i.e., 70%) as compared to all other storage or atmospheric conditions. The apparent lack of MAP effect on virus survival at lower CO_2 concentrations could be viewed as a positive outcome, since commercially distributed lettuce is stored under lower CO_2 (15%) concentrations than used in this study. However, further research is needed to address virus survival in MAP environments similar to those used in the food industry, incorporating a wider variety of foods, the effect of resident microflora of lettuce on HAV under map conditions, as well as other foodborne viruses. This is particularly useful in view of the fact that more and more varieties of foods, such as fresh-cut fruits and vegetables, are being marketed under MAP environments.

Acknowledgements

Appreciation is extended to Mr Stephen Hayward, Bureau of Biostatistics and Computer Applications, Health Canada, Ottawa, Ontario, Canada, for performing the statistical analysis of the data.

References

Anon. (1991) *The Packer*. Issue December 28, 1991.

Brody, A. L. (1995) A perspective on MAP products in North America and Western Europe. In *Principles of Modified Atmosphere and Sous Vide Product Packaging*. (Eds J. M. Farber and K. Dodds) pp. 13–37. Lancaster, Basel Technomic Publishing Co. Inc.

Cliver, D. O. (1985) Vehicular transmission of hepatitis A. *Public Health Rev.* **13,** 235–292.

Cliver, D. O. (1997) Virus transmission via foods. *Food Technol.* **51,** 71–78.

Dodds, K. (1995) Modified Atmosphere Packaging: In *Principles of Modified Atmosphere and Sous Vide Product Packaging*. (Eds J. M. Farber and K. Dodds) pp. 1–12. Lancaster, Basel Technomic Publishing Co. Inc.

Enfors, S. O. and Molin, G. (1981) The effect of different gases on the activity of different microorganisms. In *Psychrotrophic Microorganisms in Spoilage and Pathogenicity*. (Eds T. A. Roberts, G. Hobbs, J. H. B. Christian, N. Skovgaard) pp. 335–343. London, Academic Press.

Farber, J. M. (1991) Microbiological aspects of modified-atmosphere packaging technology — a review. *J. Food Prot.* **54,** 58–70.

Genigeorgis, C. A. (1985) Microbial and safety implications of the use of modified atmospheres to extend the storage life of fresh meat and fish. Int. J. *Food Microbiol.* **1,** 237–251.

Goodburn, K. E. and Halligan, A. C. (1988) Modified atmosphere packaging. A Technology guide. In*: Food Focus*. the British Manufacturing Industries Research Association. August Issue, pp 1–44.

Hanlin, J. H, Evancho, G. M. and Slade, P. J. (1995). Microbiological concerns associated with MAP and Sous Vide products. In: *Principles of Modified Atmosphere and Sous Vide Product Packaging*. (Eds J. M. Farber and K. Dodds) pp. 1–12. Lancaster, Basel, Technomic Publishing Co. Inc.

Hintlian, C. B. and Hotchkiss, J. H. (1987) Comparative growth of spoilage and pathogenic microorganisms on modified atmosphere-packaged cooked beef. *J. Food Prot.* **54,** 213–223.

Jaykus, L. (2000) Detection of human enteric viruses in foods. In: *foodborne disease handbook, volume 2: viruses, parasites, pathogens and HACCP*. (Ed S. Sattar) pp. 137–163. New York, Marcel Dekker.

Knipe, D. M (1996) Virus-Host-Cell Interactions. In: *Fields Virology, 3rd edition*. Volume 1. *Edited by* B. N. Fields, D. M. Knipe, P. M. Howley, R. M. Chanock, J. L. Melnick, T. P. Monath, B. Roizman and S. E. Straus pp. 293–316. New York, Lippincott-Raven Press.

Konowalchuk, J. and Speirs, J. I. (1975) Survival of enteric viruses on fresh vegetables. *J. Milk Food Technol.* **38,** 469–472.

Lefevre, D. 1991. The effects of modified atmospheres upon microorganisms. *Proceedings of the International Conference on Modified Atmosphere Packaging. 15th–17th October 1990.* pp. 1–13. Stratford-upon-Avon, UK: Campden Food and Drink Association.

Martens, M. and Baardseth, P. (1987) Sensory quality, In: *Postharvest Physiology of Vegetables*. (Ed J. Weichmann) pp. 427–454, New York, Marcel Decker Inc.

Mbithi, J. N., Springthorpe, V. S. and Sattar, S. A. (1991) Effect of relative humidity and air temperature on survival of hepatitis A virus on environmental surfaces. *Appl. Environ. Microbiol.* **57,** 1394–1399.

Mbithi, J. N., Springthorpe, V. S., Boulet, J. R. and Sattar, S. A. (1992) Survival of hepatitis A virus on human hands and its transfer on contact with animate and inanimate surfaces. *J. Clin. Microbiol.* **30,** 757–763.

Miller, R. G. (1966) *Simultaneous Statistical Inference*. McGraw-Hill, Toronto.

Sattar, S. A., Springthorpe, V. S., Karim, Y. and Loro, P. (1989) Chemical disinfection of non-porous inanimate surfaces experimentally contaminated with four human pathogenic viruses. *Epidemiol. Infect.* **102,** 493–505.

Searle, S. R., Speed, F. M. and Miliken, G. A. (1980) Populations marginal means in the linear model; an alternative to least squares means. *The American Statistician*. **34,** 216–221.

Weichmann, J. (1986) The effect of controlled atmosphere storage on the sensory and nutritional quality of fruits and vegetables. *Hort. Rev.* **8,** 101–127.

Zagory, D. (1995) Principles and practice of modified atmosphere packaging of horticultural commodities. In: *Principles of Modified Atmosphere and Sous Vide Product Packaging*. (Eds J. Farber and K. Dodds) pp. 175–206. Lancaster, Basel, Technomic Publishing Co. Inc.

Zagory, D. and Kader, A. A. (1988) Modified atmosphere packaging of fresh produce. *Food Technol.* **42,** 70–77.