Postharvest Seminar

Postharvest Technology Innovation Center
SUT

Postharvest Technology for Fresh-Cut Produces

by

Dr. Pascal Delaquis
Agriculture & Agri-Food Canada
Pacific Agri-Food Research Centre
Summerland, British Columbia
Canada

August 20, 2008
Suranaree University of Technology
Nakhon Ratchasima, Thailand

Pascal Delaquis, Ph.D.

Research Scientist, Food Microbiology

Contact Information:

HIGHWAY 97
SUMMERLAND, BRITISH COLUMBIA V0H 1Z0
Telephone: (250) 494-6367
Fax: (250) 494-0755
Email: delaquisp@agr.gc.ca

Areas of Expertise:

- Ecology and antimicrobial resistance of foodborne pathogens in horticultural crop production
- Ecology of foodborne pathogens in fresh-cut fruit and vegetable products
- Microbiological quality of fresh-cut fruits and vegetables
- Natural antimicrobials from plants and their use in food preservation
- Wine fermentations

Current Projects:

- Design, development, engineering and evaluation of low temperature thermal treatment for horticultural products, optimized for quality and safety. Development and evaluation of novel processing equipment capable of producing high quality minimally
- Studies on the risk of dissemination and fate of foodborne pathogens in field vegetables
- Assessment and management of biological and chemical contaminants carried in livestock and poultry manure. [GAPS]
- Methodologies to ensure the microbial safety of fresh fruits and vegetables throughout the distribution system

Affiliations:

- Adjunct Professor, University of British Columbia

Recent Publications:

2007:

- Husnik, J.I., Delaquis, P.J., Cliff, M.A., and Van Vuuren, H.J.J. (2007), "Functional Analyses of the Malolactic Wine Yeast ML01.", American Journal of Enology and Viticulture, 58(1), pp. 42-52.

2006:

- Delaquis, P.J., Wen, A., Toivonen, P.M.A., and Stanich, K. (2006), "Evidence of an antilisterial factor induced by wounding of iceberg lettuce tissues.", Letters in Applied Microbiology, 42, pp. 289-295.
- Dallaire, R., LeBlanc, D.I., Tranchant, C.C., Vasseur, L., Delaquis, P.J., and Beaulieu, C. (2006), "Monitoring the microbial populations and temperatures of fresh broccoli from harvest to retail display.", Journal of Food Protection (JFP), 69(5), pp. 1118-1125.
- Dallaire, R., Vasseur, L., LeBlanc, D.I., Tranchant, C.C., and Delaquis, P.J. (2006), "A methodological approach for assessing the microbial contamination of fresh produce from harvest to retail.", Food Protection Trends (FPT), 26(4), pp. 218-225.
- Toivonen, P.M.A. and Delaquis, P.J. (2006), "Low volume sprays to treat fresh-sliced apples with anti-browning solution.", HortTechnology, 16, pp. 257-261.
- Moon, K.D., Delaquis, P.J., Toivonen, P.M.A., and Stanich, K. (2006), "Effect of vanillin on the fate of Listeria monocytogenes and Escherichia coli O157:H7 in a model apple juice medium and in apple juice.", Food Microbiology, 23(2), pp. 169-174.
- Moon, K.D., Delaquis, P.J., Toivonen, P.M.A., Bach, S.J., Stanich, K., and Harris, L.J. (2006), "Destruction of Escherichia coli O157:H7 by vanillic acid in unpasteurized juice from six apple cultivars.", Journal of Food Protection (JFP), 69, pp. 542-547.
- Ngarmsak, M., Delaquis, P.J., Toivonen, P.M.A., Ngarmsak, T., Ooraikul, B., and Mazza, J. (2006), "Microbiology of fresh-cut mangoes prepared from fruit sanitized in hot chlorinated water.", Food Science and Technology International, 12(2), pp. 95-103.
- Ngarmsak, M., Delaquis, P.J., Toivonen, P.M.A., Ngarmsak, T., Ooraikul, B., and Mazza, J. (2006), "Antimicrobial Activity of Vanillin against Spoilage Microorganisms in Stored Fresh-Cut Mangoes.", Journal of Food Protection (JFP), 69(7), pp. 1724-1727.

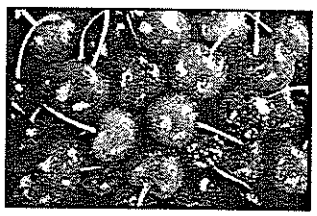
2005:

- Ngarmsak, M., Ngarmsak, T., Ooraikul, B., Delaquis, P.J., Toivonen, P.M.A., and Mazza, J. (2005), "Effect of sanitation treatment with heated, chlorinated water on microbiology of fresh-cut Thai mangos.", Acta Horticulturae (ISHS), 682, pp. 1895-1900.
- Delaquis, P.J. (2005), "Fresh-cut vegetables.", in Sapers, G.M., Gorny, J.R., Yousef, A.E. (eds.) - Microbiology of Fresh Fruits and Vegetables, CRC Press.
- Delaquis, P.J., Stanich, K., and Toivonen, P.M.A. (2005), "Effect of pH on the Inhibition of Listeria spp. By Vanillin and Vanillic Acid.", Journal of Food Protection (JFP), 68(7), pp. 1472-1476(5).

Origin and dissemination of *E. coli* in sweet cherry production systems

Pascal Delaquis
Pacific AgriFood Research Centre
Summerland, British Columbia

Sour cherries: products



Baking ingredients
(pie fillings, glazes etc.)

Jams, jellies, candies

Juices and syrups

Cosmetics

Sweet cherries: fresh market

Many varieties:

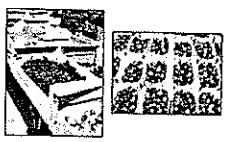
Varieties developed in Summerlafid:

Lapins
Sweetheart
Skeena

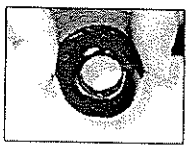
Cherry distribution and marketing

- Shipped in boxes with plastic liners

- Mainly sold in bulk or in plastic



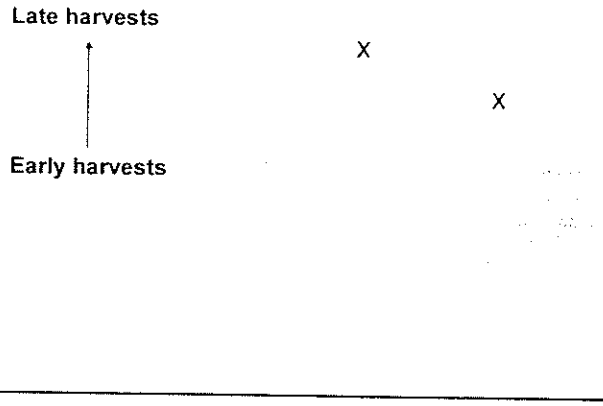
- Under development: fresh-cut cherries



Value of Canadian cherry exports, $ can

	2003	2004	2005	2006	2007
United States (U.S.)	850228	1251013	1375167	3754299	7910428
Taiwan (Taipei)	1083498	3248950	5931214	6907405	3972670
United Kingdom (U.K.)	4034600	4046937	1817830	2909737	2962762
France	788628	932089	571149	480283	1263751
Belgium	1664711	1170489	406596	848011	1081056
Netherlands	942056	1850784	1662675	2327004	1061542
Hong Kong	270722	117565	314333	154886	848672
Germany	1118429	1168724	952438	934428	558003
Thailand	5040	93113	306154	240913	534353
Spain	0	315172	138695	251242	447551
OTHERS	613645	642231	632911	430991	625169
TOTAL (ALL COUNTRIES)	11371557	14837067	14109162	19239199	21265957

Source of data: Statistics Canada
Report Date: 24-Jul-2008



Phytosanitary Regulation of the Entry of Fresh Fruits and Vegetables into the United States Cherries: U.S. import-eligible countries; world production and exports.

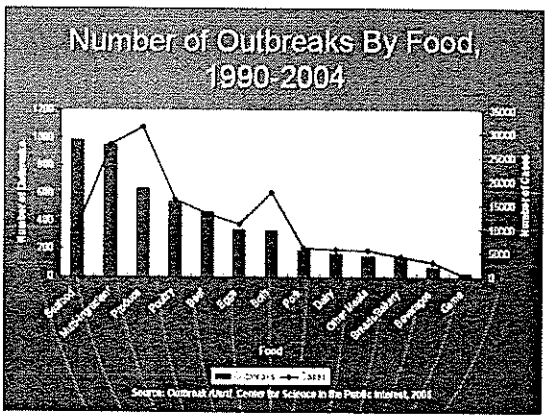
Country	Production	Total exports	Export value
	1,000 metric tons		*1,000 US$*
Argentina	6.70	2.26	5,480
Australia	8.20	1.48	10,011
Canada	6.92	2.76	11,656
Chile	33.00	16.59	61,342
Mexico	0.00	0.00	1
New Zealand	1.60	1.10	9,226

Source of data: USDA Economic Research Service
http://www.ers.usda.gov/Data/FruitVegPhyto/#2008-8-11

Food safety and cherries

- No evidence of outbreaks linked to consumption of cherries.

- The role of fresh produce in foodborne illness has increased:



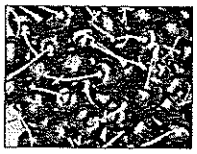
Food safety assurance systems in fruit production

- Essential to maintain markets.

- Generic models based on Good Agricultural Practices or Hazard Analysis Critical Control Point (HAACP).

- Critical control points are not always clear.

- Microbiological examination of the chain can identify critical control points.

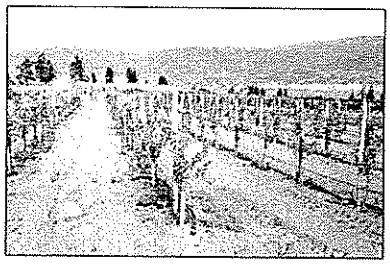


Cherry production in British Columbia: inputs and GAPs

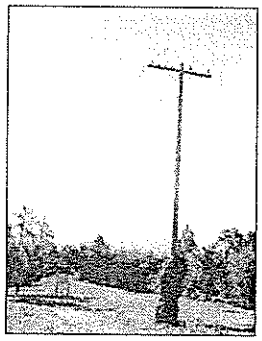
- Water: most growers use treated water, combination of drip and overhead irrigation.

- Fertilizer: mainly applied with water (fertigation).

- No manure used to produce fresh market fruit.



Pests:

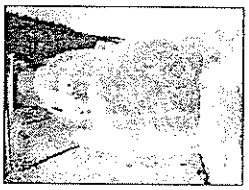


Main control method:

- Netting.

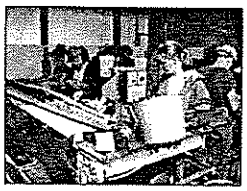
- Other methods: traps, shooting

Typical sweet cherry production system



Production

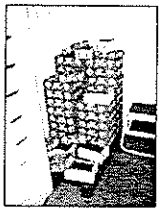
Harvest



Sorting

Hydro-cooling

Sorting, sizing



Packaging, cooling

Microbiological examination of cherry production systems to determine critical control points

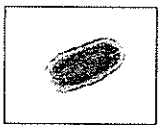
Options:

- Specific pathogens: low probability of detection.

- Indicator microorganisms from animal feces:

  Coliforms: broad group, analytical requirements low.

  E. coli: analysis more difficult but more specific, individual strains can be tracked using molecular methods.



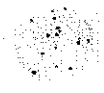
Microbiological examination of cherry production systems to determine critical control points

Year 1: survey of hydro-cooler water and fruit from random cherry packing houses.

Methods:

- Measure coliform and *E. coli* populations in water samples using a filtration method on Chromocult™ coliform agar.

- Detect *E. coli* in fruit samples by enrichment in EC broth, followed by plating onto MacConkey agar.

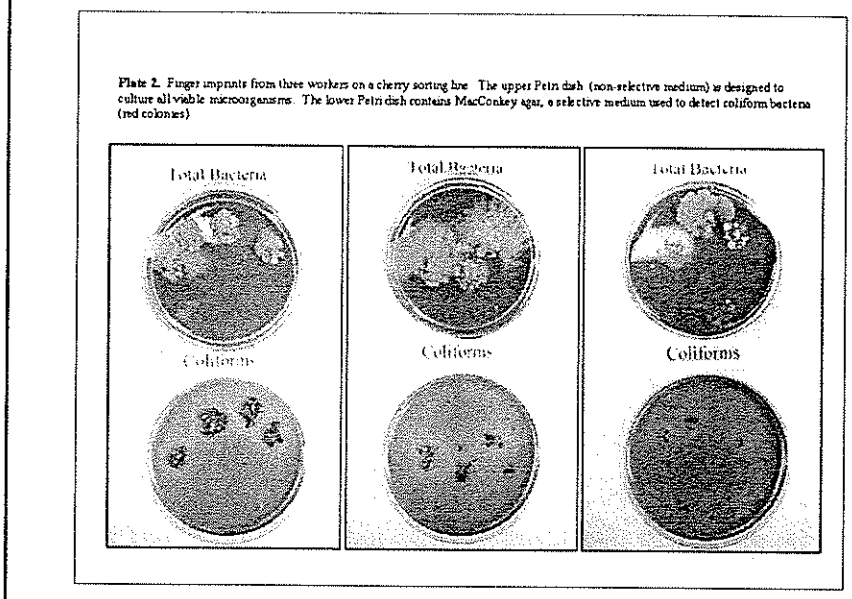


Coliforms and *E. coli* (per 100 ml) in hydro-cooler water and recovery of *E. coli* from cherries in random packing houses.

Packing house	Coliforms	*E. coli*	*E. coli* on cherries
A	140	<1	-
B	<1	<1	-
C	<1	<1	+
D	37600	30	+
E	>100,000	<1	+
F	>100,000	<1	+
G	<1	<1	-
H	>100,000	<1	-
I	<1	<1	-
J	<1	<1	+
K	<1	<1	-

Coliforms, *E. coli* (per 100 ml) and chlorine levels in process waters from a cherry packing house (20 ppm Cl target).

	DumpTank		Sorting / Sizing Tank		Hydro-cooler		
Time	Coliforms	*E. coli*	Coliforms	*E. coli*	Coliforms	*E. coli*	Free Cl
Day 1							
Start	3	<1	<1	<1	<1	<1	10
1.25 hours	>100,000	26	<1	<1			
2.25 hours	>100,000	200	1	<1	<1	<1	4
3.25 hours	>100,000	180	<1	<1			
4.25 hours	>100,000	130	10	<1	<1	<1	12
6.25 hours	>100,000	270	2	<1	<1	<1	12
8 hours	>100,000	400	1	<1	<1	<1	10
Day 2							
Start	4	<1	<1	<1	<1	<1	15
2 hours	27,600	900	<1	<1	<1	<1	16
4 hours	>100,000	1600	<1	<1	<1	<1	16
6 hours	>100,000	2100	<1	<1	<1	<1	7
8 hours	400,000	1000	<1	<1	<1	<1	14
9 hours	200,000	1600	<1	<1	<1	<1	13



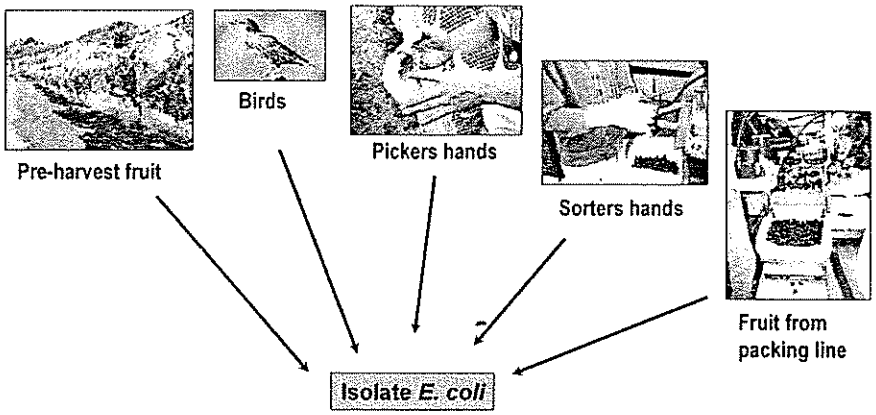
Observations from year 1

- *E. coli* on fruit is not rare

- *E. coli* accumulates in water (dump tank)
20 ppm chlorine (free) is sufficient to prevent this.

- Source of the *E. coli* unclear

Year 2: determine source and movement of *E. coli* in cherry production systems



Genotype to identify origin (s) and track specific strains

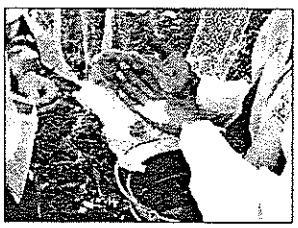
Year 2: determine source and movement of *E. coli* in cherry production systems

Methods:

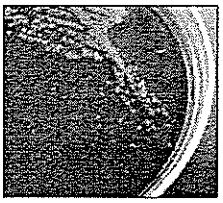
- Repeated random samplings of fruit before harvest.

- Rectal swabs of downed birds.

- Hands swabs performed on pickers in the field and sorters in the packinghouse.



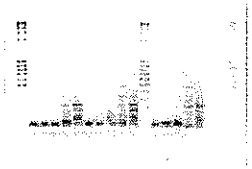
- *E. coli* in fruit samples, rectal swabs and hand swabs were recovered by enrichment in EC broth, followed by plating onto MacConkey agar.



- Isolates from each sample were purified and genotyped using Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR).

Purify isolate
Extract DNA

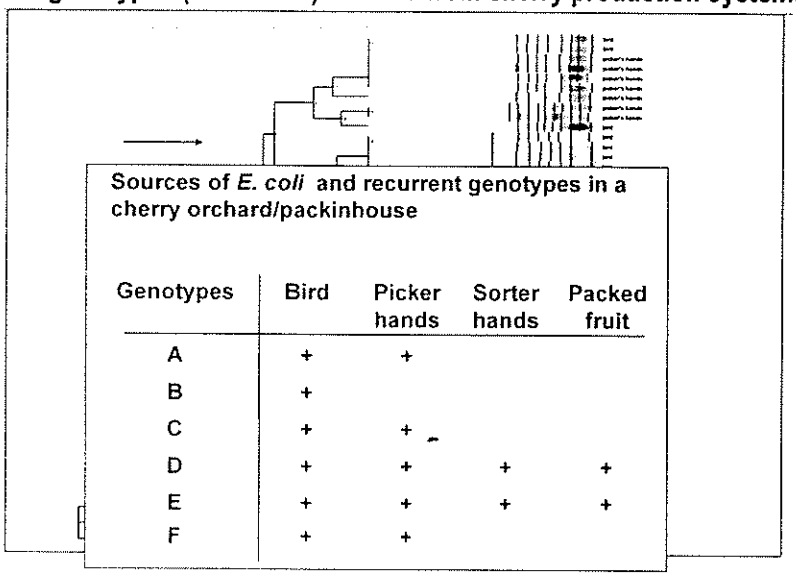
- ERIC elements are repetitive sequence elements in the enterobacterial genome. PCR primers targeting the repeats are used to "fingerprint" bacterial species or strains.



Source and percentage of samples positive for *E. coli* in a cherry orchard/packinghouse

	Fruit on the tree	Birds	Pickers' hands	Sorters' hands	Fruit from packing line	Dump tank
# samples	70	30	70	80	69	2
# positive	0	26	25	12	16	2
% positive	0	86.6	35.7	15.0	23.2	100

E. coli genotypes (ERIC-PCR) isolated from cherry production systems



Sources of *E. coli* and recurrent genotypes in a cherry orchard/packinhouse

Genotypes	Bird	Picker hands	Sorter hands	Packed fruit
A	+	+		
B	+			
C	+	+		
D	+	+	+	+
E	+	+	+	+
F	+	+		

Summary of findings:

Sources of *E. coli*

Birds | Birds, hands | Birds, more hands

Spread of *E. coli*

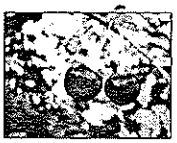
Heterogeneous distribution → Homogeneous distribution

Implications of the findings

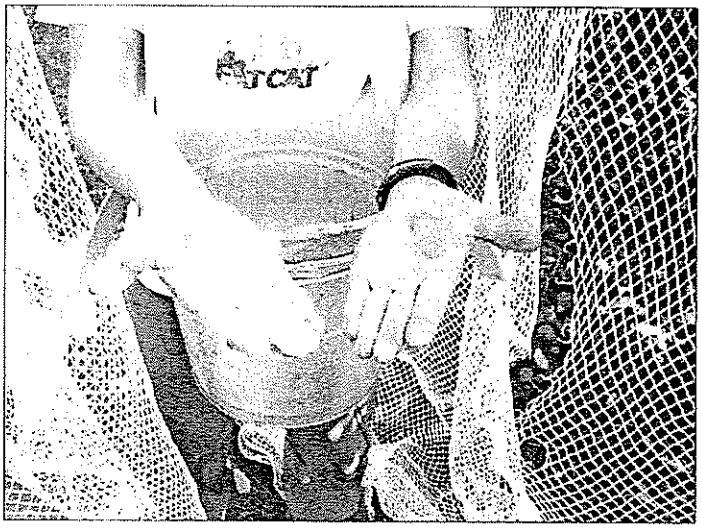
- Bird control is important for quality, yield and food safety – CCP.

- All water that comes in contact with fruit can spread contaminants and must be sanitized – CCP.

- Contact with hands spreads microbial contaminants, hand sanitation is a CCP.



Solutions for hand sanitation in the field . ?



Cherry picker's hands, 10 minutes after washing

Thank you very much for your attention!

Questions?

Factors affecting the survival of human pathogens in leafy vegetables

Pascal Delaquis
Pacific AgriFood Research Centre
Summerland, British Columbia

Outbreaks of food borne illness linked to leafy vegetables

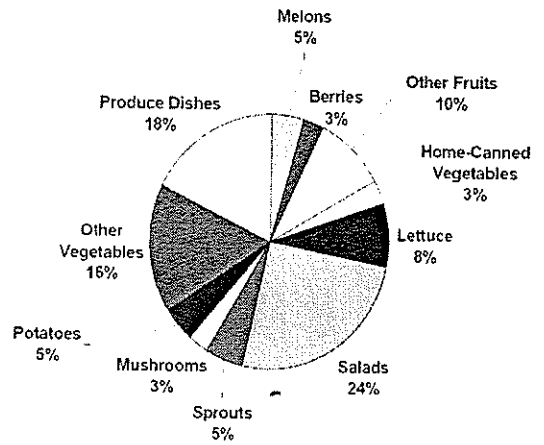
- USA 1973-2006: 502 outbreaks, 18,242 illnesses, and 15 deaths associated with "leafy greens" including lettuce, cabbage, mescalin mix, spinach or a salad item containing one or more of these leafy vegetables.

- Increase in the consumption of leafy green vegetables and the proportion of outbreaks attributed to leafy greens in the USA (1986-2005).

	% increase compared with previous decade	
	Consumption	Proportion of total outbreaks
1986-1995	17.2	59.6
1996-2005	9.0	38.6

Herman et al, 2008. http://www.cdc.gov/ncidod/EID/announcements/iceid_2008.htm.

Vehicles of produce-related outbreaks, 1990-2003

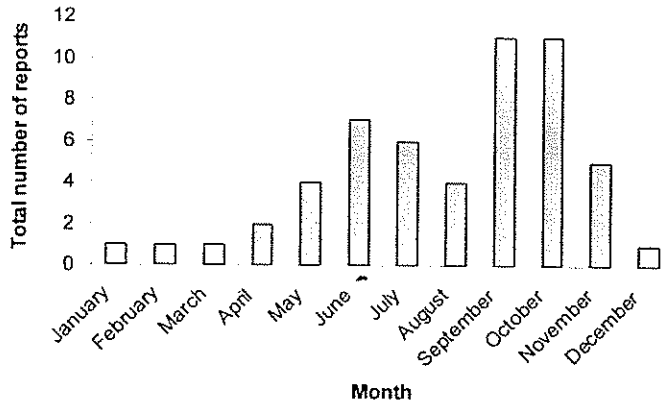


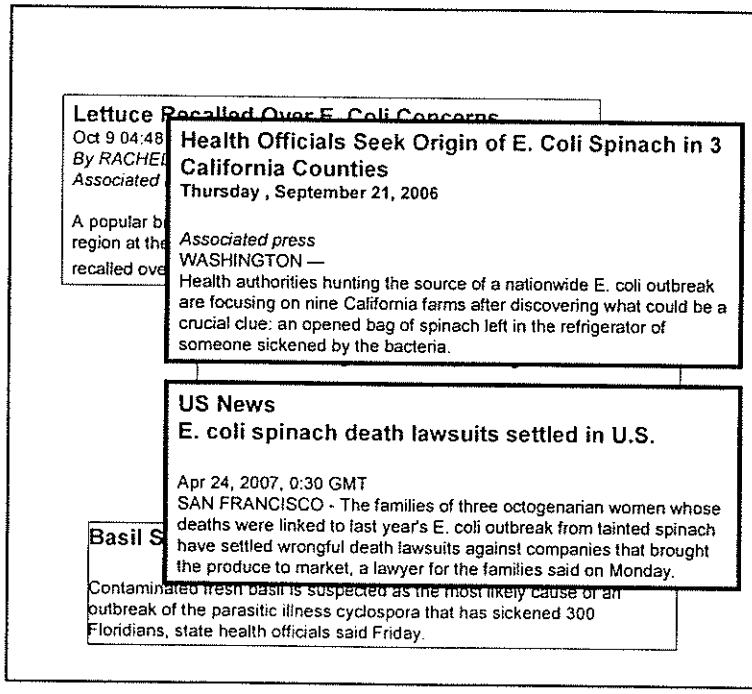
Microbial hazards associated with leafy vegetables and herbs

Bacteria	
Campylobacter jejuni	lettuce
Clostridium botulinum	cabbage
Shiga-toxigenic *Escherichia coli* (including O157)	coleslaw, lettuce, spinach, parsley
Listeria monocytogenes	cabbage (coleslaw), lettuce
Salmonella	lettuce, cabbage (coriander
Shigella sonnei	lettuce, parsley
Staphylococcus aureus	leafy vegetables (Brazil)
Vibrio cholerae	cabbage
Yersinia pseudotuberculosis	lettuce
Parasites	
Cyclospora cayetanensis	lettuce, basil
Giardia lamblia	lettuce
Fasciola hepatica	watercress
Viruses	
Calicivirus	leafy vegetables
Hepatitis A	lettuce, watercress
Norovirus, SRSV	cabbage , watercress
Rotavirus	leafy vegetables

Compiled from Harris et al. Comprehensive Reviews in Food Science and Food Safety. 2S:78-141.
EU data: http://ec.europa.eu/food/fs/sc/scf/out125_en.pdf

Seasonality of outbreaks linked to leafy vegetables in the US





5 faces. 5 agonizing deaths. 1 year later.

Spinach recall improved food safety - but is it enough?

By Elizabeth Weise and Julie Schmit
USA TODAY, Sept 21, 2007.

Ruby Trautz was the first to die.
On Aug. 27, 2006, the 81-year-old Nebraska woman was rushed to the hospital. She was in so much pain that morphine was administered. Four days later, she succumbed to a food-borne infection later identified as a virulent strain of E. coli.

The outbreak would ultimately cost the leafy green industry more than $350 million as the nation turned away from its growing appetite for fresh, ready-to-eat spinach. It's an appetite that has not returned: Sales of packaged spinach are still off about 20% from pre-outbreak levels, industry executives say.

Impact:

- Direct costs : $350 million – recall, legal fees, etc.

- Sales of spinach remain lower than before the outbreak due to lower consumer confidence

Gov. Schwarzenegger Boosts Funding for Food Safety, Response to E. coli like Threats, Takes Additional Action to Protect Produce

01/10/2007 GAAS:019:07 FOR IMMEDIATE RELEASE

To increase California's capacity to effectively respond to foodborne causes of illnesses such as E. coli, Governor Schwarzenegger is proposing to add $2.1 million to the budget of the Department of Public Health. The Department will be established in July by legislation that was approved by the Governor last year.
This week, the Schwarzenegger Administration will also hold a hearing in Monterey to work with the agriculture industry to finalize an agreement that will improve the safe handling of leafy green produce, like lettuce and spinach. California Food and Agriculture (CDFA) Secretary A.G. Kawamura has invited all stakeholders to participate in the hearing on Friday.

"Spinach" report highlights

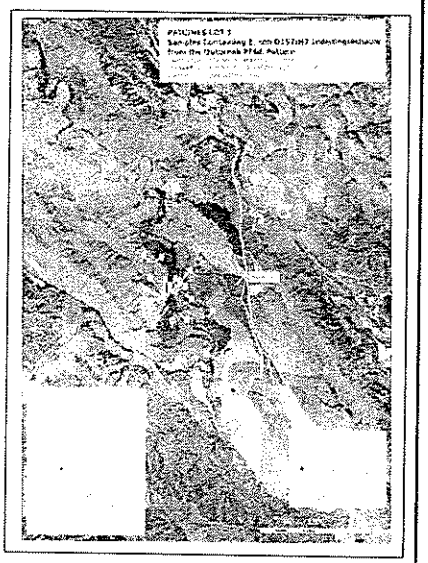
- Suspicion focused on one ranch.

- Implicated strain was not recovered from the field, well, harvesting equipment, processing plant.

- Was recovered from cattle, wild pig feces, soil, water about 1.5 km away.

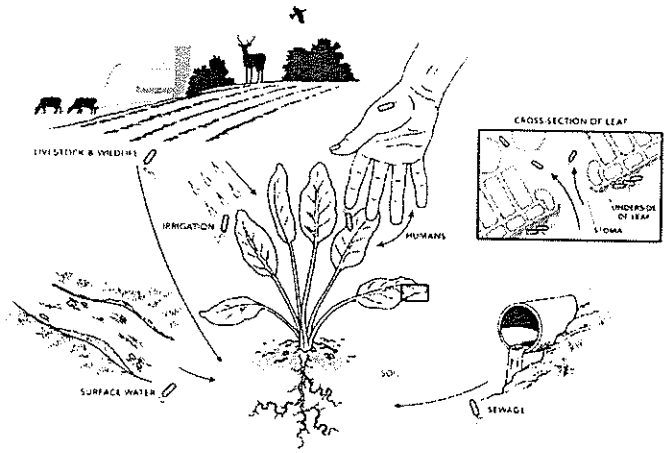
Main conclusion

- How *E. coli* O157:H7 contaminated spinach in this outbreak remains unknown.

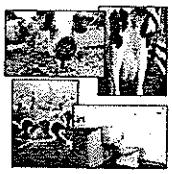


http://www.dhs.ca.gov/ps/fdb/HTML/Food/EnvinvRpt.htm

Contamination during production: a general model



Fecal oral route of transmission in an agricultural setting



Pre-harvest

Vectors
Agronomic and environmental factors

Post-harvest

Harvest
Storage
Processing
Distribution

Sufficient numbers of microorganisms to cause disease

The agricultural environment vs intestines of warm blooded animal

	Intestines	Agricultural environment
Temperature	Constant	Extremes of heat, cold
Water availability	Constant	Periods of dessication, rehydration
Nutrients	Constant, ample	Inconsistent
UV radiation	None	Occasionally intense

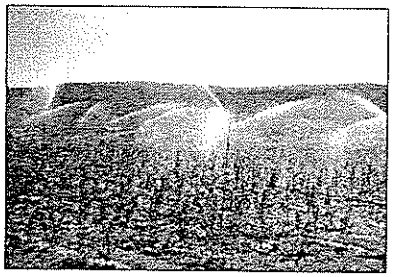
- Passage in the agricultural environment is a time of intense stress and many die. Are survivors more infective or virulent? There is some evidence this may be the case with *Salmonella* or *E. coli* O157:H7.

Water quality and irrigation

- Evidence from past outbreaks shows that plants can become contaminated via irrigation water.

- Pathogens survive for variable periods of time and can be carried over great distances.

- Influence of irrigation method on survival of pathogens is uncertain.



Water quality and irrigation

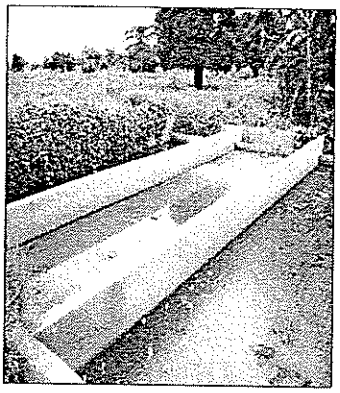
- It is increasingly clear that pathogen survival is greater is sediments than in standing or free flowing water.

- A good example of simple water management to reduce the risk of contamination with coliform bacteria and helminth eggs:

Keraita et al. 2007. Effect of low-cost irrigation methods on microbial contamination of lettuce irrigated with untreated wastewater. Tropical Medicine and International Health.12:2, p. 15 22.

The strategy:

Allow sediments to settle for a few days before use.
Draw water from the surface to irrigate.

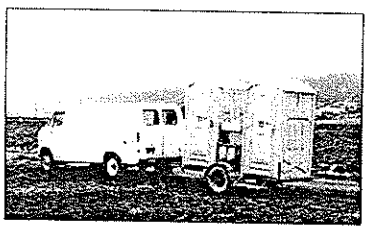


Catchment basin to allow settling

Drawing water from the surface

Human activity / animal agriculture

Not good

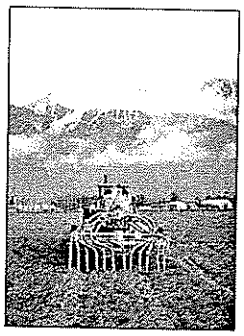


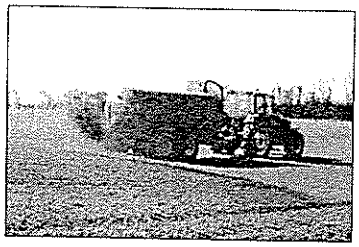
Much better

- How far away should animals be?
A lot of on-going research.

Soil quality / use of manures

- How long do pathogens survive in soil?
- How does soil type influence survival?
- How does climate influence survival?
- Does the method of application affect survival?





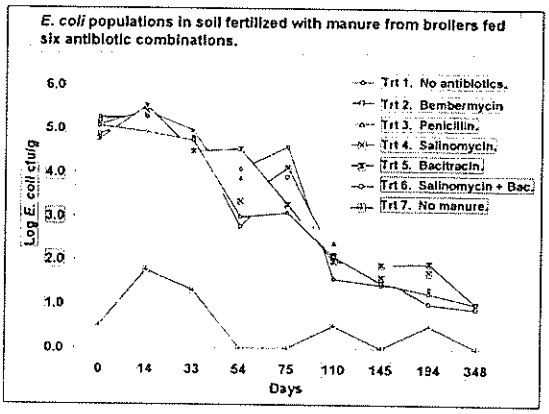
General observations on the fate of pathogens in manured soil

- Pathogen type. Survival of protozoa, helminth eggs highest, followed by bacteria and viruses. There are exceptions.
- Type of manure. Survival appears highest with cattle manures (liquid, solid), lowest with poultry litters.
- pH. Less survival in high pH manures.
- Storage conditions: survival decreases over time, anaerobic conditions favor survival.
- Temperature: better survival at lower temperatures.
- Nutrient status: survival lower in high nitrogen soils.
- Application method: tilling or injection in te soil appears to lessen survival.
- Native microbial community: lower survival in soils with high biological activity.

Example:

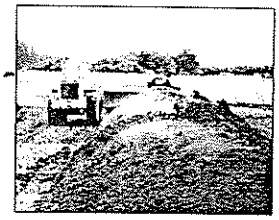
- *E. coli* from poultry manure can survive for up to 1 year in pasture soil.

- *E. coli* was recovered from grass for 1 year.



Solutions:

- Time (ageing)
- Effective composting
- Heat (or other) treatments



Research on the transfer of *E. coli* to lettuce

Objectives:

- Influence of timing, method of application on survival in soil and risk of transfer to Romaine lettuce.

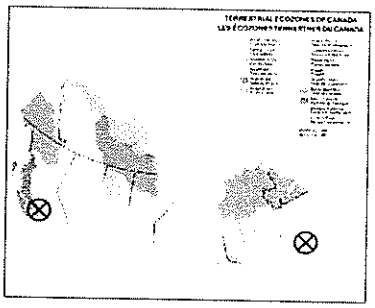
- Examine survival in fresh-cut lettuce.

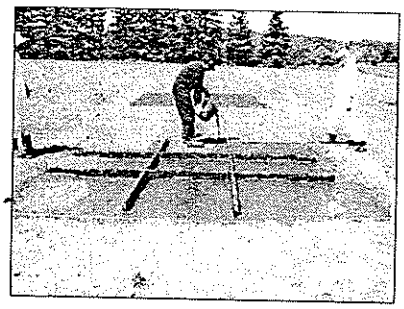
Design:

- Surface application or tilling of liquid dairy manure, 160 kgN/acre.

- Genotype isolates (PFGE), virulence genes (*stx 1, stx 2, eaeA and hlyA*).

Same protocols but at opposite end of the country ..





Analytical difficulties in field research

Sampling issues:

- Large sampling areas.

- Is the distribution of contaminants homogeneous? Is statistically sound sampling possible?

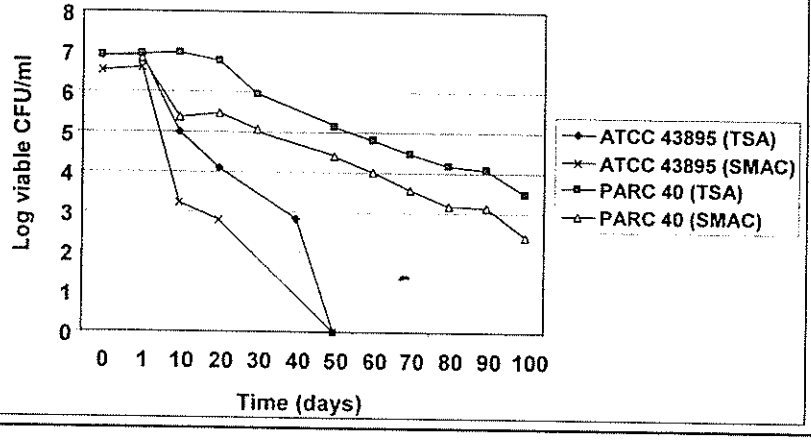
Methods for detection of pathogens:

- Are existing methods suitable for field research?

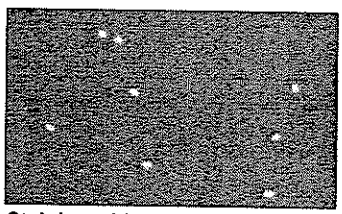
- Do they detect patogens which have been subjected to stresses induced by starvation, exposure to uv, dessication?

Do stressed bacterial pathogens enter the viable but non-culturable (VBNC) state?

Culturability of two *E. coli* O157:H7 strains on Tryptic Soy and Sorbitol MacConkey agars upon starvation in distilled water.

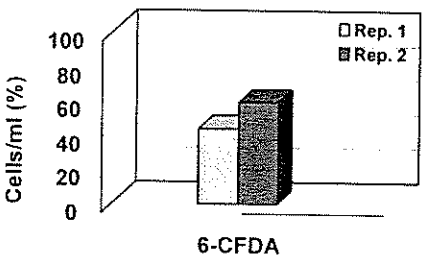


Do stressed bacterial pathogens enter the viable but non-culturable (VBNC) state?



Staining with carboxyfluorescein diacetate to detect viability.

% viable *E. coli* O157:H7 ATCC 43895 cells after 50 days starvation.



- These results suggest that stressed pathogens may not be detected using existing methods.

The "internalization" debate

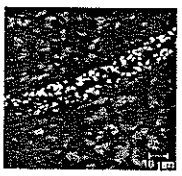
- Experimental approaches

Add pathogen to seed, soil or plant

"Sterilize" surface using Cl, $HgCl_2$, etc.

Analyze tissues

Examine by fluorescence or confocal scanning laser microscopy



Confocal scanning laser micrograph of fluorescent green *E. coli* gaining access to the xylem of cut leaf lettuce.

The "internalization" debate

- Experimental evidence is contradictory. For example:

Posters presented at the International Association of Food Protection Conference, August 2008, Columbus, Ohio, USA	Internalization
Potential Internalization of *Escherichia coli* O157:H7 in Lettuce (*Lactuca sativa L.*) by Soil Inoculation	No
Potential Internalization of *Escherichia coli* O157:H7 in Pre-Harvest Iceberg Lettuce (*Lactuca sativa L.*)	No
Pre-Harvest Internalization of Zoonotic Pathogens by Lettuce as Influenced by Environmental Growth Conditions	No
Visualization of Attachment and Internalization of a Bioluminescent Derivative of *Escherichia coli* O157:H7 ATCC 43895 on Lettuce Leaves	Yes
Transfer Prevalence of *Escherichia coli* O157:H7 from Soil, Water, and Manure Contaminated with Low Numbers of the Pathogen to Lettuce Plants of Varying Age	Yes

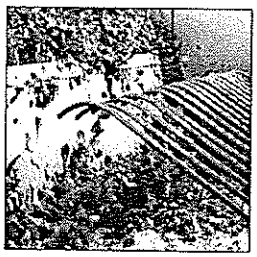
- Important to note: all of these experiments were done in growth chambers of greenhouses.

Processed (fresh-cut) produce

Harvest, cool
↓
Trim, pare
↓
Cut or slice
↓
Wash (Cl, O_3, H_2O_2 etc.)
↓
Dehydrate
↓
Package, MAP
↓
Store

Stresses associated with processing

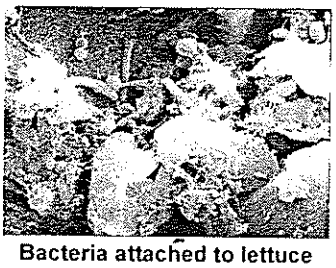
- Exposure to sanitizers.
- Low temperature.
- Change in atmosphere.



- Again, some pathogens may die but many can survive processing.

Effect of processing on survival in fresh-cut leafy vegetables

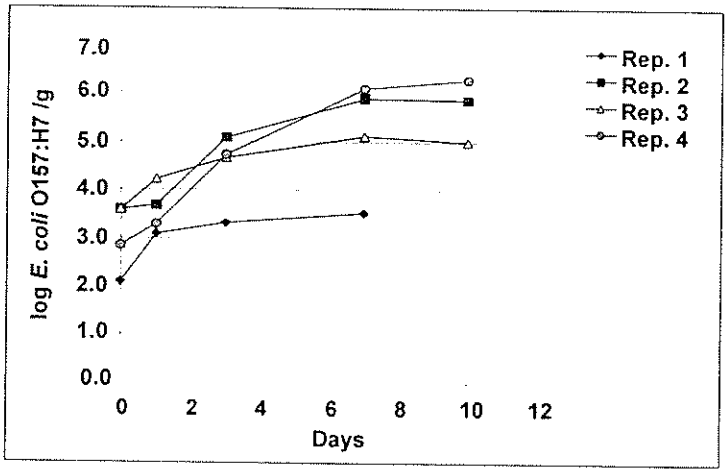
- Current sanitizers or sanitary treatments (Cl, O_3, H_2O_2) are not very effective against pathogens. Microorganisms attached to the leaf surface or buried in the waxy cuticle remain protected.



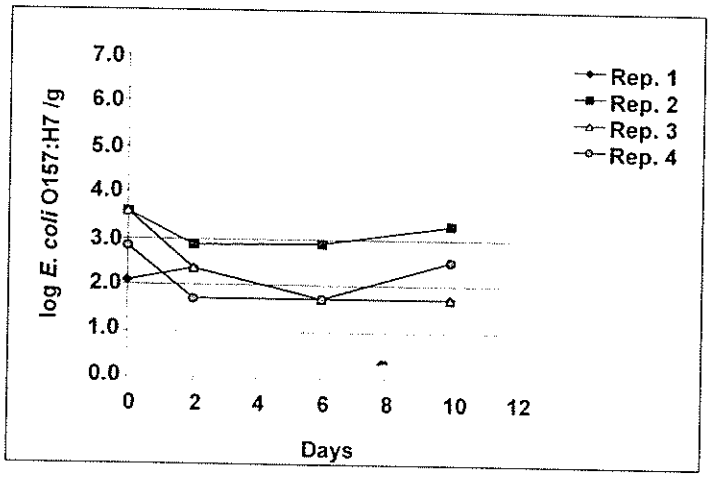
Bacteria attached to lettuce

- There is ample evidence that bacterial pathogens can grow in packaged leafy vegetables

Example: effect of temperature on the fate of *E. coli* O157:H7 in fresh-cut Romaine lettuce stored at 15° C.



Example: effect of temperature on the fate of *E. coli* O157:H7 in fresh-cut Romaine lettuce stored at 8° C.



Growth of bacterial pathogens in stored fresh-cut leafy green vegetables

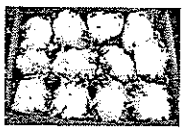
- Some unanswered questions:

  Do modified atmospheres inhibit or stimulate growth?

  Is competition with spoilage bacteria important?

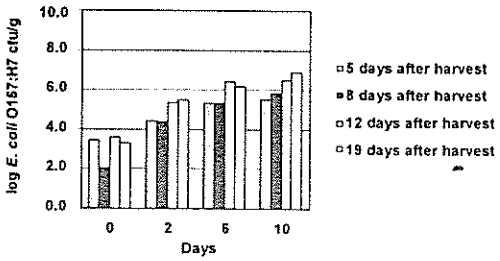
  How do bacterial pathogens interact with plant tissues?

Effect of raw material "age" on the fate of *E. coli* O157:H7 in fresh-cut lettuce

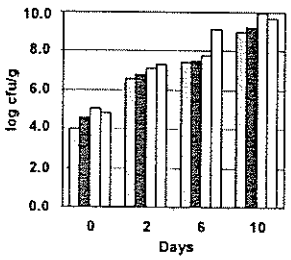


Store at 1° C For various periods of time → Process, add *E. coli* O157:H7 5 strain mix → Store

Growth of *E. coli* O157:H7 in packaged Romaine lettuce stored at 15°C

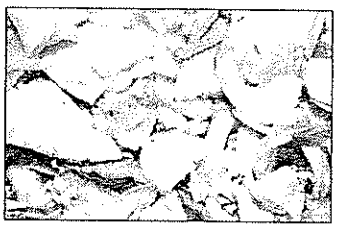


Total aerobic microbial populations in packaged Romaine lettuce stored at 15°C

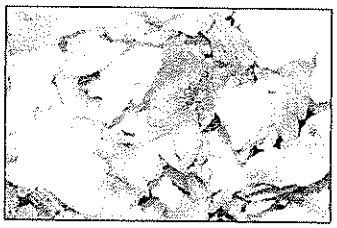


Interactions between bacterial pathogens and plant tissues

Effect of warm water treatments on the quality of fresh-cut lettuce

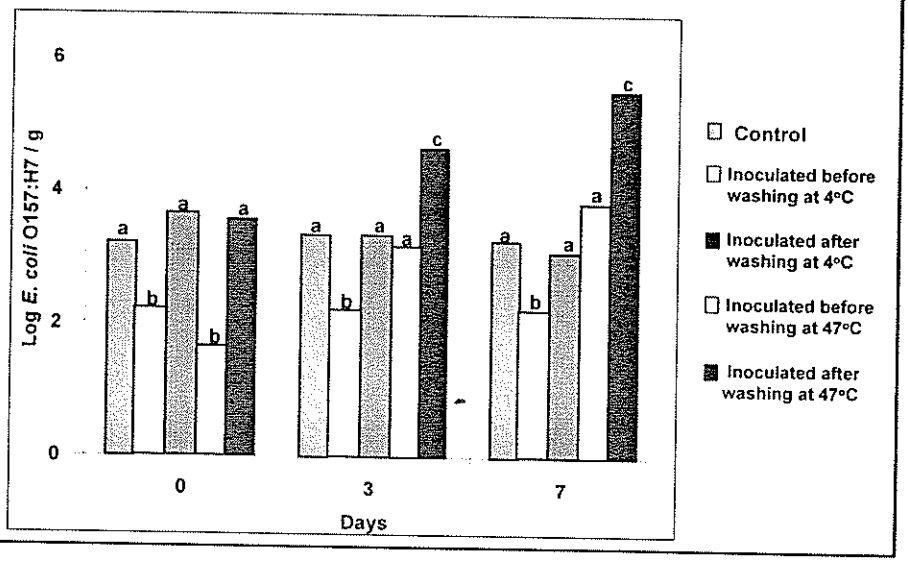


Control



Washed at 47°C, 3 min, 100 ppm Cl

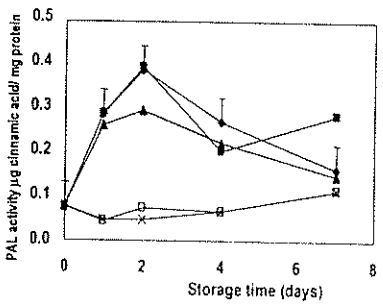
Effect of wash treatments on the fate of *E. coli* O157:H7 in packaged iceberg lettuce stored at 10° C



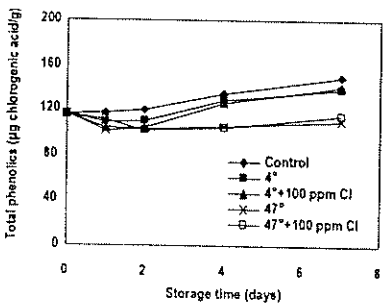
Effect of heat treatments on the physiology of lettuce

phenylalanine —Phenylalanine ammonia-lyase (PAL)→ cinnamic acid ····→ brown colored derivatives

Phenylalanine ammonia lyase (PAL) activity in iceberg lettuce tissues stored at 5° C washed at two temperatures.



Total phenolics in iceberg lettuce tissues stored at 5° C washed at two temperatures.



Wound induced phenyl propanoid metabolism in lettuce tissue

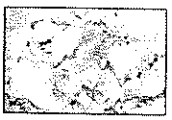
Phenylalanine → (Phenylalanine ammonia-lyase (PAL)) → Cinnamic acid

Cinnamic acid → Coumaric acid, Caffeic acid, Ferulic acid, Chlorogenic acid

Coumaric acid, Caffeic acid, Ferulic acid, Chlorogenic acid → Derivatives of tartaric acid, quinic acid

Derivatives of tartaric acid, quinic acid → Many other derivatives, condensed brown tannins

Effect of browning on the growth of pathogens

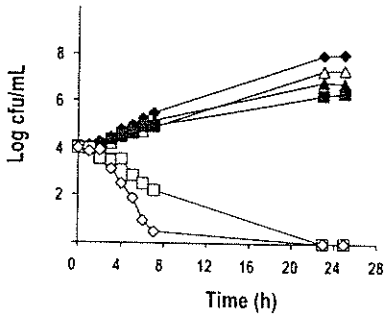


Cut iceberg lettuce

No wash
Wash at 4°C
Wash at 47°C

Package, store at 4° C for 3 days

Prepare sterile lettuce extract
Add *Listeria monocytogenes*
Incubate at 25° C



Interactions between bacterial pathogens and plant tissues

- These results suggest that physiological reactions in the tissues may influence the survival of pathogens.

- Plant pathologists know a lot about interactions between plants and microorganisms. New to food microbiologists .

- Are antimicrobial systems in plant tissues inactivated by handling or processing of leafy vegetables?

Summary

- Much remains to be learned about:

  The interaction between pathogens and plant tissues.

  The influence of variety, maturity, post-harvest handling and processing on physiological reactions that influence the fate of human pathogens in leafy vegetables.

- Factors that influence quality impact on safety. A systems based approach to the production and processing of leafy green vegetables is highly desirable.

Thank you very much for your attention!

Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

Evidence of an antilisterial factor induced by wounding of iceberg lettuce tissues

P.J. Delaquis, A. Wen, P.M.A. Toivonen and K. Stanich

Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, BC, Canada

Keywords
antilisterial factor, iceberg lettuce, *Listeria monocytogenes*.

Correspondence
Pascal J. Delaquis, Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, 4200 Highway 97 South, Summerland, BC V0H 1Z0, Canada. E-mail: delaquisp@agr.gc.ca

2004/1356: received 25 November 2004, revised 2 June 2005 and accepted 3 June 2005

doi:10.1111/j.1472-765X.2005.01826.x

Abstract

Aims: To examine the influence of wound-associated reactions in cut iceberg lettuce (*Lactuca sativa* L.) tissues on the fate of *Listeria monocytogenes*.

Methods and Results: Aqueous extracts prepared from shredded iceberg lettuce before and after storage in high oxygen permeability film were inoculated with *L. monocytogenes*. *Listeria monocytogenes* grew in extracts prepared from fresh lettuce. In contrast, inhibition ranging from arrested growth to a decline in cell viability was observed in extracts prepared from samples stored for 1–3 days. Similar behaviour was evident in lettuce shreds inoculated with 10^5 CFU g^{-1} *L. monocytogenes* immediately after processing or after 3 days in storage. Heat treatment of the cut tissues at 47°C for 3 min before storage diminished the inhibitory effect.

Conclusions: The results provided evidence that an antilisterial factor or factors are released by wounded iceberg lettuce tissues. Antilisterial activity was mitigated by heat treatment of the lettuce.

Significance and Impact of Study: This study indicates that intrinsic factors associated with plant metabolism could play a significant role in the ecology of human pathogens in packaged horticultural products.

Introduction

Although it is well established that the psychrothrophic foodborne pathogen *Listeria monocytogenes* can grow in refrigerated, packaged fresh-cut iceberg lettuce, behaviour of the species in this ecosystem remains poorly understood. Some reports describe growth stimulation postulated to result from alterations in the size and possibly the composition of the spoilage microflora in response to selective pressures induced by modified atmospheres (Steinbruegge *et al.* 1988; Farber *et al.* 1998; Jacxsens *et al.* 1999). Conflicting observations on the effects of modified atmospheres are also found in the scientific literature however. Growth patterns for *L. monocytogenes* inoculated onto lettuce stored in air and under anoxic atmospheres were identical in a study by Beuchat and Brackett (1990). Furthermore, attempts to verify the competition hypothesis by co-inoculation with variable populations of native micro-organisms have been inconclusive. For example, Francis and O'Beirne (1997) found that survival *L. innocua* was unaffected by background populations ranging from 10^3 to 10^7 CFU g^{-1}.

The maintenance of oxygen-depleted atmospheres and low storage temperatures reduces enzymatic browning in packaged lettuce. Despite these measures, shelf-life is restricted by the appearance of discolourations and additional means to alleviate the problem are under investigation. A potential solution involves the application of mild heat treatments or heat shocks before packaging (Delaquis *et al.* 1999, 2002; Loaiza-Velarde *et al.* 1997; Loaiza-Velarde and Saltveit 2001; Saltveit 2000). Temperatures between 47 and 50°C for 90–180 s delay the onset of browning by several days in refrigerated product. In addition, such temperatures are lethal to micro-organisms associated with the plant surface and population reductions 1–2 log CFU g^{-1} lettuce higher than those achieved with conventional washes in cold chlorinated water have been reported (Delaquis *et al.* 1999). Unfortunately, the

© 2006 Agriculture and Agri-Food Canada, Letters in Applied Microbiology 42 (2006) 289–295

perceived advantages of heat treatments are tempered by evidence of accelerated microbial growth during subsequent refrigerated storage. Faster development of the spoilage microflora has been observed and inoculation with *L. monocytogenes* confirmed that growth is enhanced by prior heat treatment of the lettuce (Li *et al.* 2002; Delaquis *et al.* 2002).

Tissue wounding is unavoidable during processing of lettuce into fresh-cut products. Wounding induces numerous physiological alterations including elevated respiration rates, ethylene synthesis, oxidative browning, wound healing reactions, secondary metabolite synthesis and water loss (Brecht 1995; Cantos *et al.* 2001; Kang and Saltveit 2003). The appearance of browning is associated with phenylpropanoid metabolism, a series of reactions that can, with the intervention of polyphenol oxidase, lead to the formation and accumulation of brown phenolic compounds (Camm and Towers 1973). *De novo* synthesis of phenylalanine ammonia lyase (PAL) under aerobic conditions and PAL catalyzed deamination of L-phenylalanine to trans-cinnamic acid and ammonia is the first step in a series of reactions referred to as the phenylpropanoid pathway. Heat shock (Fukumoto *et al.* 2002) and modified atmospheres (López-Gálvez *et al.* 1996) both inhibit PAL activity and the accumulation of phenylpropanoid metabolites in cut lettuce tissues. The role of these reactions in the ecology of microbial spoilage or the fate of foodborne pathogens in plant foods is not known. Enhanced growth of *L. monocytogenes* in cut lettuce subjected to heat shocks or storage under modified atmospheres hints that such treatments may weaken intrinsic barriers to growth. Moreover, the ability to modulate wound-associated reactions by heat shock may provide a useful experimental tool for the study of putative interactions between plant tissues and colonizing micro-organisms. This approach was exploited in the present investigation on the interaction between *L. monocytogenes* and lettuce tissues.

Materials and methods

Preparation of lettuce shreds and lettuce extracts

Experiments were carried with shreds or aqueous tissue extracts prepared from whole lettuce (*Lactuca sativa* L. type Salinas) heads obtained from a local retailer. The latter were grown in California and were delivered to the retail outlet approx. 3 days after harvest. Outer and damaged leaves were discarded and shreds (3 × 20 mm) were cut from both photosynthetic and vascular tissues using a stainless steel knife. Where necessary, the shreds were packed in bags (30 × 17 cm) made of PD941 film (Cryovac, Mississauga, ON, Canada; oxygen transmission rate 16 544 ml O_2 m^{-2} 24 h^{-1}) that were closed with 2-mm wide seals using a SwissVac vacuum sealer (SwissVac, Luzern, Switzerland). Aqueous extracts were prepared by placing 50 g of shreds in 250-ml Erlenmeyer flasks (VWR, Edmonton, AB, Canada) with 100 ml sterile distilled water, followed by agitation on an orbital shaker at 125 rpm for 1 h at room temperature. The extracts were then filtered through Whatman no. 4 filter paper (Whatman, Florham Park, NJ, USA), spun at 10 000 *g* for 30 min at 4°C in a centrifuge (RC 5B Plus; Sorvall, Thermo Electron, Asheville, NC, USA) and sterilized by passing through 0·22 μm membranes (Supor 200; Millipore, Billerica, MA, USA).

Microbiological methods

Experiments were carried out with *L. monocytogenes* strain CFAR 92 (graciously provided by Dr R. McKellar, AAFC, Guelph, ON, Canada). The strain was originally isolated from chicken wiener and is sensitive to the inhibitory effects of phenolic acids (Wen *et al.* 2003). Stock cultures were maintained at 4°C on Trypticase Soy Agar (BBL; BD Biosciences, Mississauga, ON, Canada) amended with 5 g l^{-1} Yeast Extract (TSYA; Oxoid, Nepean, ON, Canada). Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to 10 ml Trypticase Soy Yeast Extract Broth (TSYB; BD Biosciences), followed by incubation overnight at 30°C. Inocula were prepared by centrifugation of the culture at 3220 *g* for 15 min at room temperature. Sterile distilled water (10 ml) was added to re-suspend the cells by gentle agitation in a laboratory mixer and the tubes were spun anew at 3220 *g* for 15 min. The density of the final washed cell suspension was adjusted with sterile distilled water in a spectrophotometer (620 nm) to obtain approx. 5×10^6 CFU ml^{-1}.

Cell populations in cultures or aqueous lettuce extracts were determined by spreading appropriate dilutions prepared in 0·1% (w/v) peptone onto TSYA, followed by incubation at 30°C for 48 h. Where enrichment was necessary for the detection of cell densities below the limit of detection, 10 ml of the extract were added to 100 ml TSYB. Enrichment medium fluids were spread onto PALCAM agar (Difco; BD Biosciences) after 48 h incubation at 30°C. Bacterial populations in cut lettuce were determined from homogenates prepared by pummelling 50 g lettuce with 450 ml 0·1% peptone in a Lab Stomacher (Seward, Colworth, UK). Appropriate dilutions in 0·1% peptone were spread onto Plate Count Agar (Difco) and PALCAM agar (Difco) to estimate total aerobic and *L. monocytogenes* populations respectively. Plate Count Agar plates were incubated at 30°C for 48 h in air and PALCAM plates were incubated at 30°C for 48 h in

© 2006 Agriculture and Agri-Food Canada,

anaerobic jars to discourage the growth of aerobic microorganisms. Several isolated colonies were picked from the PALCAM plates for confirmation by the latex test (*L. monocytogenes* Latex Test; Oxoid).

Fate of *L. monocytogenes* in aqueous lettuce extracts

Sterile aqueous extracts were prepared from fresh lettuce and from packaged shreds stored for 1, 2 and 3 days at 15 ± 1°C on metal shelves in a darkened incubator. One hundred millilitres of each extract were placed in 250 ml screw-capped Erlenmeyer flasks. Inoculum was added to achieve initial cell populations of approx. 10^4 CFU ml^{-1} *L. monocytogenes* and the flasks were incubated at 25 ± 1°C with agitation. Samples were periodically withdrawn to estimate population densities. Three independent trials were carried out.

Fate of *L. monocytogenes* in extracts prepared from washed and stored shreds

Lettuce shreds were prepared as before. Seven hundred grams were dipped for 3 min in a stainless steel container with 14 l of a 100 μg ml^{-1} sodium hypochlorite solution cooled to 4°C or heated to 47°C. Excess water was removed in a home salad spinner immediately after treatment and the shreds were packed and stored at 15 ± 1°C. Aqueous extracts were prepared from lettuce subjected to each treatment immediately after preparation and after 3 days in storage. Extracts inoculated with *L. monocytogenes* were incubated at 25 ± 1°C and cell populations were estimated over time.

Fate of *L. monocytogenes* in aqueous phenolic depleted lettuce extract

Phenolic compounds were removed from an aqueous extract prepared from fresh lettuce by passage through a hydrophobic Sep-Pak C18 column (Waters, Milford, MA, USA). Total phenolic content before and after passage were measured using the Folin–Ciocalteau reagent method of Singleton and Rossi (1965). Unaltered and phenolic-depleted extracts were inoculated with *L. monocytogenes* and cell populations were estimated during incubation at 25 ± 1°C.

Fate of *L. monocytogenes* in packaged lettuce shreds

Lettuce shreds were placed in film bags without further treatment and after chlorinated water washes at 4 or 47°C. One half of the packages were immediately inoculated with 10^5 CFU g^{-1} *L. monocytogenes* by direct addition of 0·5 ml of a washed cell suspension. The packages were sealed, inverted several times to mix the contents and placed in an incubator at 15 ± 1°C. The second half was stored at 15 ± 1°C for 3 days before inoculation. Inocula were added through small openings made with sterile scissors, which were closed with a handheld sealer before returning the packages to the incubator. Two packages from each treatment combination were analysed daily over 3 days to determine changes in total aerobic and *L. monocytogenes* populations. The experiment was repeated three times.

Results and discussion

The experimental strain chosen for this work grew well in aqueous extracts prepared from fresh lettuce obtained on three separate occasions (Fig. 1). In contrast, declining cell densities indicative of inhibitory activity were evident in all extracts prepared from shreds stored at 15 ± 1°C for 1, 2 or 3 days. The effect was always more pronounced in extracts made from lettuce stored for >1 day and was strongest in lettuce stored for 2 days, in two out of three trials. In both cases cell densities were below the limit of detection for the plating assay (10 CFU ml^{-1}) after 2–3 h incubation and attempts to recover viable cells after 25 h incubation were unsuccessful. These observations provided evidence that a water soluble factor or factors lethal to *L. monocytogenes* accumulated in cut lettuce tissues stored under aerobic conditions. Aqueous extracts were also prepared from cut lettuce subjected to a variety of physical treatments to determine whether the appearance of inhibitory activity was affected by heat-induced physiological alterations. As shown in Fig. 2, strong inhibition of *L. monocytogenes* was apparent from declining cell densities in extracts prepared from lettuce stored for 3 days without treatment or following a wash in cold chlorinated water, a common practice in the fresh-cut industry. The consequences of heat treatment before storage were not as clear. Effects ranged from growth inhibition without loss in cell viability (trial I), no inhibition (trial II) and extensive cell death (trial III). These apparent discrepancies were postulated to arise from variability in raw materials because of varietal, seasonal, agronomic or postharvest handling, all factors known to affect physiological reactions in plant tissues.

Evidence of a time-dependant release of inhibitory factor (s) by cut tissues invited speculation about a possible role for such reactions in the ecology of *L. monocytogenes* in stored, packaged lettuce. The fate of the species was consequently examined in shreds inoculated with 10^5 CFU g^{-1} immediately after processing or after 3 days in storage. Figure 3 shows total aerobic and *L. monocytogenes* populations after 3 days storage at 15 ± 1°C. *Listeria monocytogenes* populations remained relatively

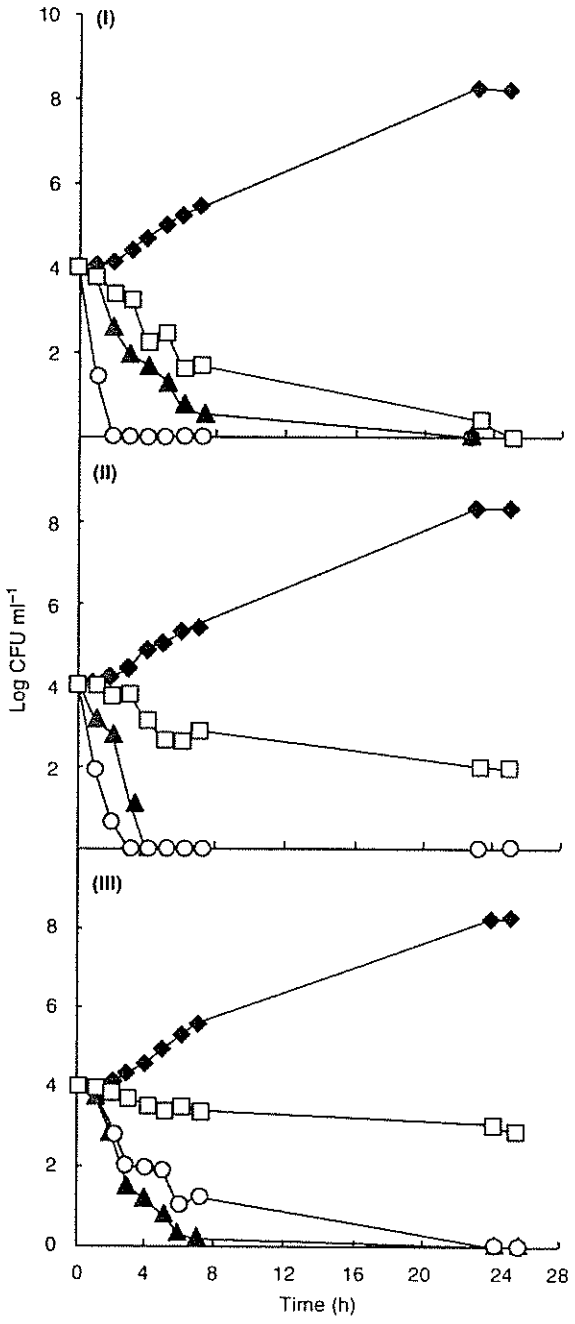


Figure 1 Fate of *Listeria monocytogenes* in aqueous extracts prepared from packaged iceberg lettuce shreds stored aerobically for 0 day (◆), 1 day (□), 2 days (○) and 3 days (▲) at 15°C respectively. Results of three experiments (I, II and III) are shown.

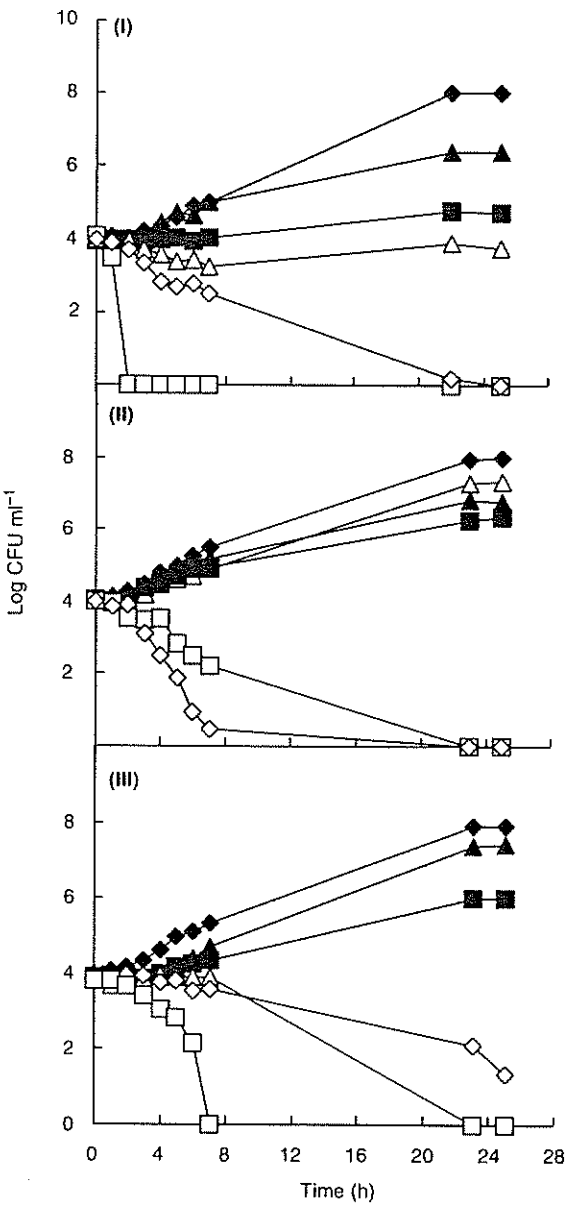


Figure 2 Fate of *Listeria monocytogenes* in aqueous extracts prepared from packaged iceberg lettuce shreds stored aerobically at 15°C after a 3-min wash in chlorinated (100 μg ml^{-1} NaOCl) water. Extracts prepared from unwashed lettuce stored at day 0 (◆) and after 3 days (◇); lettuce washed at 4°C at day 0 (■) and after 3 days storage (□) or at 47°C at day 0 (▲) and after 3 days (△). Results of three experiments (I, II and III) are shown.

unchanged in untreated lettuce shreds inoculated immediately after packaging. In contrast, a population decline was evident in shreds inoculated after 3 days in storage, an observation that hinted at possible attenuation by the background microflora as the latter accounted for a considerably larger portion of the total aerobic microbial population. However, results obtained with lettuce subjected to the wash treatments indicated that competition

© 2006 Agriculture and Agri-Food Canada,

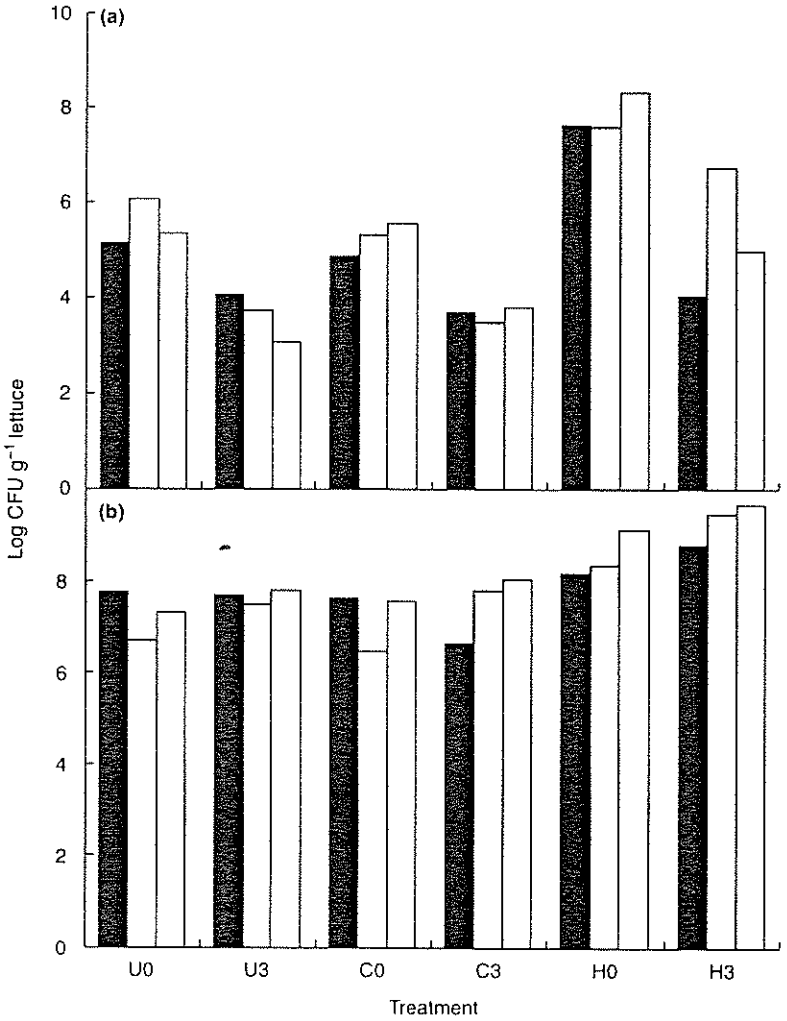


Figure 3 *Listeria monocytogenes* (a) and total aerobic microbial populations (b) in packaged lettuce shreds stored for 3 days at 15°C after inoculation. Treatments: U0 and U3, untreated lettuce inoculated after one and three days at 15°C; C0 and C3, inoculated after one and three days at 15°C following a 3-min wash at 4°C in chlorinated (100 μg ml^{-1} NaOCl) water; H0 and H3, inoculated after 1 and 3 days at 15°C following a 3-min wash at 47°C in chlorinated water. Results of three replicate experiments are shown.

alone could not account for the fate of the test strain. Washing in cold chlorinated water prior to inoculation had no effect on the fate of *L. monocytogenes* despite immediate reductions in background microbial populations of 1–2 log CFU g^{-1} (data not shown). Furthermore, the test strain grew rapidly in lettuce subjected to a heat shock despite background populations of log 8–9 CFU g^{-1}. Hence intrinsic physiological alterations mitigated by heat shocks prior to packaging clearly influenced the behaviour of *L. monocytogenes* in packaged cut lettuce. Mild heat shocks are known to decrease PAL activity and the synthesis of phenolic compounds that accumulate in wounded lettuce tissues, including cinnamic, chlorogenic, isochlorogenic, caffeic, caffeoyltartaric and dicaffeoyl tartaric acids (Ke and Saltveit 1988, 1989; Peiser *et al.* 1998; Fukumoto *et al.* 2002). Interestingly, several of these compounds exhibit antimicrobial activity *in vitro* (Barber *et al.*, 2000; Wen *et al.* 2003). The contribution of phenolic compounds to the inhibitory effect of cut lettuce tissue was verified by inoculation of the experimental strain into a phenolic-depleted aqueous extract. Results reported in Fig. 4 show that removal of 95% of phenolic compounds from lettuce extract (from 13·16 mg l^{-1} expressed as chlorogenic acid to 2·62 mg l^{-1} after separation) did not prevent inhibition of *L. monocytogenes*.

The inhibitory principle elicited in cut iceberg lettuce tissue under aerobic conditions remains unidentified and is the subject of continuing investigations in our laboratory. A phenolic nature has not been ruled out as some of the less hydrophobic chemical species in this complex group of phytochemicals may not be retained on a C18 column. In addition, several have well-defined functions in the defence of plants against microbial infection,

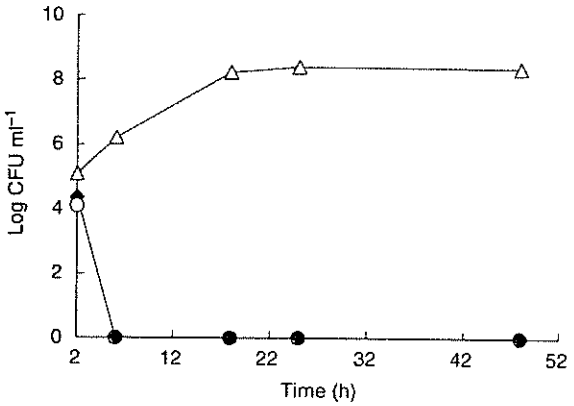


Figure 4 Fate of *Listeria monocytogenes* in aqueous extracts prepared from fresh lettuce shreds (△), after 3 days storage at 15°C before (◆) and after passage through a C18 column.

although comparatively little is known about their role in lettuce. One notable exception is the phytoalexin lettucenin A, a sesquiterpene lactone that is induced following microbial challenge by plant pathogens, particularly Gram-negative species such as *Pseudomonas cichorii* (Bestwick *et al.* 1995; Bennett *et al.* 2002). Whether other constitutive or induced phytoalexins exist in wounded iceberg lettuce tissues is unknown. The classical plant pathology literature describes many other classes of compounds active in plant defence mechanisms, including a range of phenolics, fatty acid derivatives, amino acid metabolites, peptides and proteins (Walker 1994). Clearly, their influence on the behaviour of human pathogens such as *L. monocytogenes* in plant foods remains to be defined. The evidence presented herein revealed the presence of a wound-associated factor with antilisterial activity that influenced the fate of the species in packaged cut lettuce. Furthermore, there were indications that treatments designed for improved quality retention may lessen the antilisterial activity. These observations point to the relevance of additional research aimed at characterization of the interaction between *L. monocytogenes* and plant foods that are distributed in a fresh-cut format. Moreover, a more complete understanding of the ecology of this psychotrophic pathogen in packaged, fresh horticultural products is desirable for the development of appropriate processing strategies to minimize the risk associated with growth during refrigerated storage.

References

Barber, M.S., McConnell, V.S. and DeCaux, B.S. (2000) Antimicrobial intermediates of the general phenylpropanoid and lignin specific pathways. *Phytochem* **54**, 53–56.

Bestwick, L., Bennett, M.H., Mansfield, J.W. and Rossiter, J.T. (1995) Accumulation of the phytoalexin lettucenin a and changes in 3-hydroxy-3-methylglutaryl coenzyme a reductase activity in lettuce seedlings with the red spot disorder. *Phytochemistry* **39**, 775–777.

Bennett, M.H., Mansfield, J.W., Lewis, M.J. and Beale, M.H. (2002) Cloning and expression of sesquiterpene synthase genes from lettuce (*Lactuca sativa* L.). *Phytochemistry* **60**, 255–261.

Beuchat, L.R. and Brackett, R.E. (1990) Survival and growth of *Listeria monocytogenes* on lettuce as influenced by shredding, chlorine treatment, modified atmosphere packaging and temperature. *J Food Sci* **55**, 755–758,

Brecht, J.K. (1995) Physiology of lightly processed fruits and vegetables. *J Hort Sci* **30**, 18–22.

Camm, E.L. and Towers, G.H.N. (1973) Phenylalanine ammonia lyase. *Phytochemistry* **12**, 961–973.

Cantos, E., Espin, J.C. and Tomas-Barberan, F.A. (2001) Effect of wounding on phenolic enzymes in six minimally processed lettuce cultivars upon storage. *J Agric Food Chem* **49**, 322–330.

Delaquis, P.J., Stewart, S., Toivonen, P.M.A. and Moyls, A.L. (1999) Effect of warm, chlorinated water on the microbial flora of shredded iceberg lettuce. *Food Res Int* **32**, 7–14.

Delaquis, P.J., Stewart, S., Cazaux, S. and Toivonen, P. (2002) Survival and growth of *Listeria monocytogenes* and *Escherichia coli* O157 : H7 in ready-to-eat iceberg lettuce washed in warm, chlorinated water. *J Food Prot* **65**, 459–464.

Farber, J.M., Wang, S.L., Cai, Y. and Zhang, S. (1998) Changes in populations of *Listeria monocytogenes* inoculated on packaged fresh-cut vegetables. *J Food Prot* **61**, 192–195.

Francis, G.A. and O'Beirne, D. (1997) Effects of gas atmosphere, antimicrobial dip and temperature on the fate of *Listeria innocua* and *Listeria monocytogenes* on minimally processed lettuce. *Int J Food Sci Technol* **32**, 141–151.

Fukumoto, L.R., Delaquis, P.J. and Toivonen, P.M.A. (2002) Effect of water wash temperature and chlorination on phenolic metabolism and browning of stored iceberg lettuce photosynthetic and vascular tissues. *J Agric Food Chem* **50**, 4503–4511.

Jacxsens, L., Devlieghere, F., Falcato, P. and Debevre, J. (1999) Behavior of *Listeria monocytogenes* and *Aeromonas* spp. on fresh-cut produce packaged under equilibrium-modified atmosphere. *J Food Prot* **62**, 1128–1135.

Kang, H.M. and Saltveit, M.E. (2003) Wound-induced increases in phenolic content of fresh-cut lettuceis reduced by a short immersion in aqueous hypertonic solutions. *Postharvest Biol Technol* **29**, 271–277.

Ke, D. and Saltveit, M.E. (1988) Plant hormone interaction and phenolic metabolism in the regulation of russet spotting in iceberg lettuce. *Plant Physiol* **88**, 1136–1140.

Ke, D. and Saltveit, M.E. (1989) Wound-induced ethylene production, phenolic metabolism and susceptibility to russet spotting in iceberg lettuce. *Physiol Plant* **76**, 412–418.

© 2006 Agriculture and Agri-Food Canada.

Li, R., Brackett, R.E., Chen, J. and Beuchat, L.R. (2002) Mild heat treatment of lettuce enhances growth of *Listeria monocytogenes* during subsequent storage at 5°C or 15°C. *J Appl Microbiol* **92**, 269–275.

Loaiza-Velarde, J.G., Tomas-Barberan, F.A. and Saltveit, M.E. (1997) Effect of intensity and duration of heat-shock treatments on wound-induced phenolic metabolism in iceberg lettuce. *J Am Soc Hort Sci* **122**, 873–877.

Loaiza-Velarde, J.G. and Saltveit, M.E. (2001) Heat shocks applied either before or after wounding reduce browning of lettuce leaf tissue. *J Am Soc Hort Sci* **126**, 227–234.

López-Gálvez, G., Saltveit, M. and Cantwell, M. (1996) Wound-induced phenylalanine ammonia lyase activity: factors affecting its induction and correlation with the quality of minimally processed lettuces. *Postharvest Biol Technol* **9**, 223–233.

Peiser, G., López-Gálvez, G., Cantwell, M. and Saltveit, M.E. (1998) Phenylalanine ammonia lyase inhibitors control browning of cut lettuce. *Postharvest Biol Technol* **14**, 171–177.

Saltveit, M.E. (2000) Wound induced changes in phenolic metabolism and tissue browning are altered by heat shock. *Postharvest Biol Technol* **21**, 61–69.

Singleton, V.L. and Rossi, J.A. (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* **16**, 144–158.

Steinbruegge, E.G., Maxcy, R.B. and Liewen, M.B. (1988) Fate of *Listeria monocytogenes* on ready to serve lettuce. *J Food Prot* **51**, 596–599.

Walker, J.R.L. (1994) Antimicrobial compounds in food plants. In *Natural Antimicrobial Systems and Food Preservation* ed. Dillon, V.M. and Board, R.G. pp. 181–204. Wallingford, UK: CAB International.

Wen, A., Delaquis, P.J. and Stanich, K. (2003) Antilisterial activity of selected phenolic acids. *Food Microbiol* **20**, 305–311.

Ascorbic Acid Retention, Microbial Growth, and Sensory Acceptability of Lettuce Leaves Subjected to Mild Heat Shocks

MARÍA DEL R. MOREIRA, ALEJANDRA G. PONCE, CARLOS E. DEL VALLE, AND SARA I. ROURA

ABSTRACT: Heat shocks not only produced a reduction in the initial content of ascorbic acid but also affected the rate of degradation of ascorbic acid during refrigerated storage. Samples treated at higher temperatures presented faster rates of degradation. The heat shocks produced an initial reduction in the counts of mesophilic aerobic microorganisms, with greater reductions associated with the higher shock temperatures. However, high shock temperatures promoted faster microbial growths during storage, probably due to the liberation of nutrients by the disruption of membrane barriers. The sensory attributes of lettuce leaves subjected to heat shocks presented mixed results. Thermal treatments affected the enzymatic activity, reducing browning phenomena, but also caused deleterious effects on surface color and texture of lettuce leaves. Heat shocks of 50 °C could be useful for short-term preservation of minimally processed lettuce, where the high rate of metabolic processes cause great deterioration of fresh products.

Keywords: heat shock treatments, lettuce, ascorbic acid, mesophilic bacteria-sensory attributes

Introduction

Consumers demand fruits and vegetables that are convenient to prepare yet maintain a fresh-like quality and contain only natural ingredients. Fresh fruits and vegetables are living tissues subject to continuous changes after harvest. Although some changes are desirable, most, from a consumer's standpoint, are not (Kader 1992). The factors that shorten the shelf life of produce are enzymatic browning, microbial spoilage, white surfaces discoloration, and senescence caused by continued respiration and ethylene production (Reyes 1996). To prolong the shelf life of fresh fruits and vegetables, the microbial population growth must be controlled and several postharvest operations, such as washing and removal of damaged tissues, are used to reduce initial counts. However, this processing affects the metabolism of the plant tissues adversely, and the shelf life of the vegetables or fruits may be shortened. Enzymatic browning, in fruits and vegetables tissues, can cause undesirable quality changes during handling, processing, and storage (Vamos-Vigyazo 1981).

Lettuce is a leafy vegetable, which is difficult to process because of its high mechanical and physiological fragility. One of the most common post-harvest disorders of this product is tissue browning. A wide array of processing and storage methods has been developed to combat these deterioration factors. A lot of them make use of synthetic additives and antioxidants. However, consumers tend to be suspicious of chemical additives, and thus the search for natural and socially more acceptable technologies has been intensified (Skandamis and others 2001). New methods to control browning reactions and to control natural microflora on lettuce include mild heat shock treatments. The ease with which a heat shock can be administered to lettuce and the lack of an offensive chemical residue make this technique an attractive alternative to preserve fresh cut lettuce (Saltveit 2000). Loaiza-Velarde and others (1997) reported that dipping lettuce in water at 45 °C to 55 °C extends the shelf life and visual quality of minimally processed lettuce by inhibiting the activity of phenylalanine-ammonia-lyase (PAL), the enzyme that initiates biosynthesis of phenolic compounds that lead to visible discoloration along the cut edge of the lettuce leaf (López-Gálvez and others 1996). Delaquis and others (1999) reported that treated lettuce with chlorinated water for 3 min at 47 °C delayed the growth of spoilage microflora by several days during storage at 4 °C. Li and others (2001) suggested that heat (50 °C) treatment may have delayed browning and reduced initial populations of some groups of microorganisms naturally occurring on iceberg lettuce, but may have enhanced microbial growth during subsequent storage.

Several reports analyzed mild heat shocks to control tissue browning (Saltveit 1998; Saltveit 2000; Loaiza-Velarde and others 2001; Murata and others 2004). However, reports on the effects of heat shocks on the microbiological and sensory properties of lettuce are scarce. Moreover, little information is available on the effects of mild heat treatments on ascorbic acid retention in lettuces leaves. Generally, mild heat shock treatments are applied together with chlorinated water. Chlorine is the primary sanitizing agent used for washing fruits and vegetable; however, it has a negative impact on the environment and occasionally, it may cause the product to have an objectionable aftertaste.

The purpose of the present study was to determine the effect of unchlorinated water heat shocks (2 min at 30 °C, 40 °C, and 50 °C) on fresh lettuce leaves. The effects after the treatments and during the subsequent refrigerated storage were assessed on ascorbic acid retention, microbial count, and sensorial acceptability.

Materials and Methods

Sample preparation

Heads of Romaine lettuce (*Lactuca sativa*, type Cos, variety Logi-

MS 20040829 Submitted 12/29/04, Revised 6/13/05, Accepted 8/9/05. *Authors Moreira and Valle are with Grupo de Investigación en Ingeniería en Alimentos, Facultad de Ingeniería, Univ. Nacional de Mar del Plata (UNMDP), Juan B. Justo 4302, CP: 7600, Mar del Plata, Provincia de Buenos Aires, Argentina. Authors Moreira, Ponce, and Roura are with Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) Buenos Aires, Argentina. Direct inquiries to author Moreira (E-mail: moreira_maria@fi.mdp.edu.ar).*

© 2006 Institute of Food Technologists
Further reproduction without permission is prohibited

folial) were harvested at optimal maturity. They were transported to the laboratory within 2 h of harvesting (in container at 5 °C) and were immediately subjected to preliminary operations and conditioning. Outer leaves were discarded and only photosynthetic leaves (green leaves) were included in the samples. To apply the heat-shock, the following steps were carried out: lettuce leaves were dipped in water baths with gentle agitation (10 L volume capacity) for 2 min at 4 temperatures (20 °C, 30 °C, 40 °C, and 50 °C) at a weight ratio 1:10. Because of mixing the samples at room temperature with the bath water, the final bath temperature decreased 0.5 °C to 3.5 °C, with the larger drops corresponding to the higher temperatures. Bath temperatures were monitored with a Data Logger (RS232 Interface, Model 8828P, U.S.A.). It was assumed that the leaves reached the bath temperature almost immediately because they were placed loose in the agitated baths, they were less than 0.5 mm thick, and they would have thermal diffusivity similar to that of water.

Afterward, lettuce leaves were dipped in a water bath at 5 °C for 30 s and then centrifuged for 30 s at 500 rpm to eliminate surface water. Samples treated in water at 20 °C were taken as the control samples. Leaves were piled up in 100-g stacks, placed in polyethylene bags (25 × 20 cm, useful volume: 1.8 L) with an O_2 permeability of 520 to 4000 $cm^3/m^2/d$, CO_2 permeability of 3900 to 10000 $cm^3/m^2/d$, and water vapor of 4 to 10 $g/m^2/d$. During sample packing, the environmental temperature was 15 °C. Samples were placed in boxes with overall dimensions of 0.4 × 0.3 × 0.3 m, made of heavy-duty 0.60-cm-thick transparent acrylic, with 97% to 99% relative humidity, and stored at 5 °C to 7 °C. Samples were evaluated periodically for up to 6 d.

Microbiological studies

Lettuce leaves (25 g) were macerated in 90 mL PO_4K_3 buffer solution (0.1 mol/L), pH = 7.2, with a homogenizer (Stomacher 400 Circulator Homogenizer). Macerated lettuce was accomplished by spread plating. The enumeration and differentiation of mesophilic aerobic bacteria were performed on (Plate Count Agar (PCA) and incubated at 35 °C for 48 h (Moreira and others 2003). Microbial counts were performed in duplicate on 2 lots from 3 independent runs.

Determination of ascorbic acid

Ascorbic acid content was determined by the titrimetric assay described by Pelletier (1985). Ground lettuce leaves (20 g) were extracted with 100 mL of metaphosphoric acid solution (60 g/kg) for 3 min using a Multiquick, MR 5550 M CA tissue homogenizer by Braun (Kronberg, Germany) with an homogenizer speed of 3500 to 7000 rpm. The homogenate was made up to 250 mL with 30 g/kg metaphosphoric acid and filtered through Whatman nr 42 filter paper. Temperature during ascorbic acid extraction was maintained at 0 °C. Aliquots (5 mL each) of the filtrate were titrated with 2,6-dichloroindophenol. Ascorbic acid contents (mg/100 g) were reported on a wet basis and were performed by triplicate on 3 lots from 3 separate experimental runs (Moreira and others 2003).

Sensory evaluation

The ability of panelists (members of our laboratory with experience in sensory evaluation of leafy vegetables) to discriminate and reproduce results was tested in replicate tests on fresh leaves treated with heat shock and not treated. Six judges, aged 30 to 45 y (4 females and 2 males, all members of the laboratory, with experience in the sensory evaluation of leafy vegetables), were trained in quality evaluation of lettuce. At each sampling time (0, 1, 2, 3, 4, 5, and 6 d of storage), lettuce leaves were removed 20 min before evaluation to be equilibrating at room temperature and subjected to sensory evaluation in replicate on 3 independent lots.

The coded (3-digit) samples were presented 1 at a time in random order to the judges who sat at a round table and made independent evaluations. Each participant was provided with a plate, a pencil, and papers for writing. Sensory sessions were conducted in an air-ventilated room under white light (daylight equivalent). The sensory attributes color (uniformity and intensity), midrib and edge browning, texture and sensorial acceptability (overall visual quality) were scored on a 5-point scale, in which 5 = very good (essentially free from defects) and 1 = poor (intensive defects) (Roura and others 2000).

Statistical analysis

Differences among samples were tested by variance analysis (Box and other 1978). Differences in slopes for ascorbic acid evolution in lettuce leaves subject to different heat temperature shocks were tested according to Volk (1980). Wherever differences are reported as significant, a 99.5% or 99.9% confidence level was used.

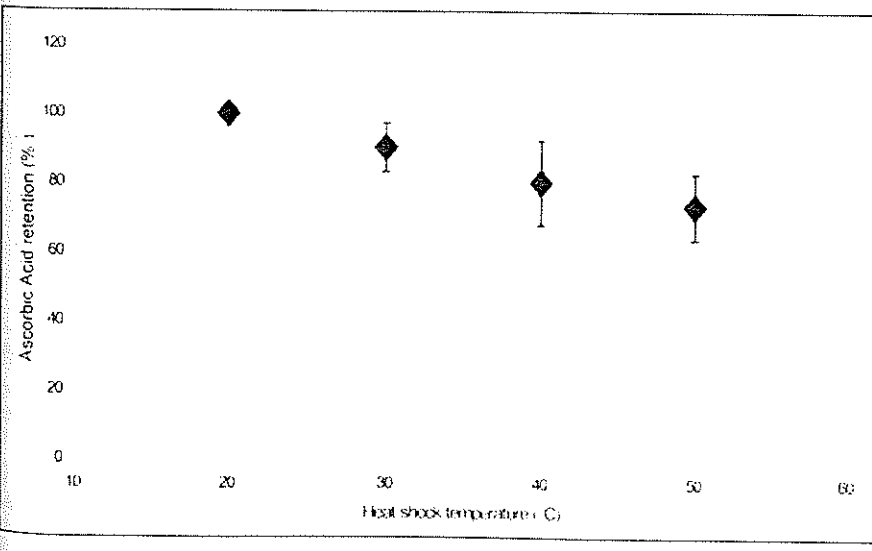


Figure 1—Percentage of ascorbic acid retention in fresh lettuce leaves influenced by heat shock temperature. Means of 15 determinations. Vertical lines represent SD. The initial ascorbic acid content of fresh leaf lettuce in control samples was 9.40 ± 1.60 (mg/100 g of fresh weight, *n* = 15).

Results and Discussion

Heat shock effects on ascorbic acid degradation and microbial populations

Ascorbic acid is heat and oxygen labile and is therefore a sensitive indicator of vitamin losses in vegetables (Logomarsino and Gao 1991). The initial ascorbic acid content of fresh leaf lettuce dipped in water baths for 2 min at 20 °C (control samples) was 9.40 ± 1.60 (mg/100 g of fresh weight, n = 15). These values were similar to those previously reported by Roura and others (2003) for lettuce leaves. Figure 1 shows the retention of ascorbic acid with reference to the control sample for the different thermal shock temperatures. Higher heat shock temperatures lead to lower ascorbic acid retention in the lettuce. At a heat shock temperature of 50 °C, the ascorbic acid reduction was almost 30% compared with control samples. These results could contradict those of Murata and others (2004) who reported that 90 s at 50 °C did not affect ascorbic acid contents of cut lettuce. However, these authors worked with cut lettuce that may have lost some ascorbic acid during the handling operations before the heat shocks, whereas the present study deals with whole leaves. Wadsö and others (2004) suggest that the intercellular voids formed during cutting could be filled with sap and thus present a higher diffusion resistance than normal tissue. This could exert a protective action against further ascorbic acid losses.

Figure 2 presents the evolution of ascorbic acid contents during refrigerated storage. If the degradation of ascorbic acid follows 1st-order kinetics, the ln of the ratio of ascorbic acid contents at time t over the initial contents should present a lineal dependence with time. Figure 2 shows the ln of that ratio during storage of the different samples and the corresponding best fit straight lines. The slopes of these tendency lines would represent the specific rate of the ascorbic acid degradation. Specific rates dependence of ascorbic acid degradation (K) with the temperature (T) of the thermal treatments can be expressed as:

$$K = 0.001T + 0.0121 \ (r^2 = 0.9927) \qquad (1)$$

Similar results for the degradation of ascorbic acid in Swiss chard after an induction period was reported in a previous work (Moreira and others 2003).

It can be seen in Figure 2 that the specific rate of ascorbic acid degradation increased (higher slopes of the tendency lines) with the temperature of the thermal treatments. Thus, a thermal shock of 2 min at 50 °C not only resulted in an initial reduction of 30% in ascorbic acid contents but also induced higher degradation rate during refrigerated storage.

Initial log counts for mesophilic aerobic microorganisms, as a consequence of the thermal treatments, were 6.43 ± 0.46 (log colony-forming units [CFU]/g, n = 16). These values were similar to those previously reported by Roura and others (2003) for the same lettuce variety. At zero d of storage, microbial counts values in samples treated with heat shocks at 30 °C, 40 °C, and 50 °C were lower than the control samples (Figure 3). Delaquis and others (2004) working with fresh-cut iceberg lettuce treated for 1 min at 50 °C in chlorinated water found microbial population reductions of 1 order log. Li and others (2001) reported higher log mesophilic aerobic bacteria reductions (1.73 to 1.96) in lettuce treated in warm (50 °C) chlorinated water during 90 s. The presence of chlorine could be responsible for the larger reductions in microbial counts with shorter temperature exposures. Figure 3 also shows the evolution of mesophilic aerobic microorganisms counts on lettuce leaves during refrigerated storage. Independent of the thermal treatment, mesophilic aerobic microorganisms did not increase during the 1st 2 d of storage. In samples immersed in water at 30 °C and 50 °C, significant reductions of mesophilic aerobic bacteria were obtained at 0, 1, and 2 d. Heat shocks at 30 °C and 50 °C did not reduce microbial counts significantly at 3, 4, and 6 d, with no significant differences between them. At the end of storage (6 d), samples that had been exposed to shock temperatures at 30 °C and 50 °C showed the highest microbial populations (3 log cycles) compared with samples treated at 20 °C and 40 °C, respectively. A possible explanation for samples treated at 50 °C would be that lettuce leaves are very sensitive to abusive temperature, even with short exposure times. These treatments would disrupt membrane physical barriers and liberate nutrients from the cells. Finally, this facilitated access to nutrients would be responsible for the greater proliferation of microorganisms. The previously described results would indicate that thermal shock treatments could help reduce the initial microbial

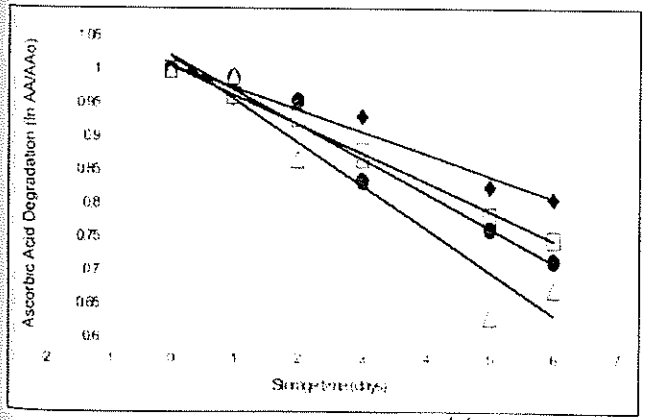


Figure 2—Ascorbic acid degradation ($\ln \frac{AA}{AAo}$) in lettuce leaves dipped in water at: 20 °C (solid diamond, ♦) $Y_{20\,°C}$ = −0.033x + 1.0063 R^2 = 0.9591, 30 °C (open square, □) $Y_{30\,°C}$ = −0.043x + 1.0055 R^2 = 0.9967, 40 °C (solid circle, ●) $Y_{40\,°C}$ = −0.051x + 1.0217 R^2 = 0.9583, 50 °C (open triangle, △) $Y_{50\,°C}$ = −0.064x + 1.0208 R^2 = 0.9187, during 6 d of storage. Each assay was performed by triplicate of 3 lots on 3 separate experimental runs.

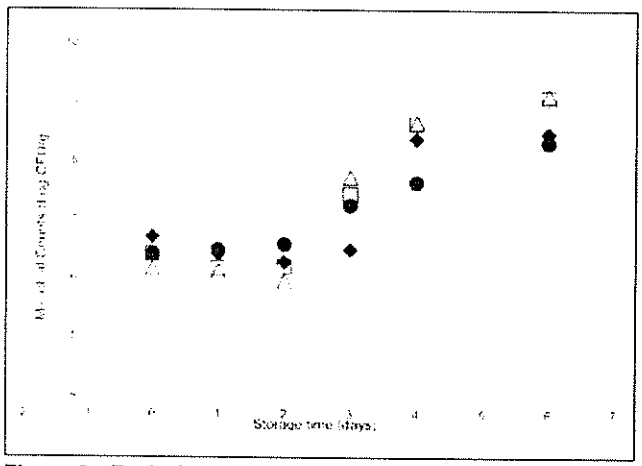


Figure 3—Evolution of mesophilic bacteria (log colony-forming units [CFU]/g) in lettuce leaves dipped in water at 20 °C (♦), 30 °C (□), 40 °C (●), and 50 °C (△) during 6 d of storage. Each assay was performed by duplicate of 2 lots on 3 separate experimental runs.

URLs and E-mail addresses are active links at www.ift.org

Table 1—Sensory attributes (midrib and edge browning, color, texture, and sensorial acceptability) as influenced by heat shock temperature and by refrigerated storage at 5 °C for up to 6 d[a,b]

Sensory attributes[c]	Heat shock temperature (°C)	Storage time (d) 0	2	4	6
Midrib browning	20	5.00a,d ± 0.00	4.50b,d ± 0.16	4.00b,d ± 0.20	2.70c,d ± 0.25
	30	5.00a,d ± 0.00	4.50b,d ± 0.19	4.50b,e ± 0.12	3.20c,e ± 0.18
	40	5.00a,d ± 0.00	5.00b,e ± 0.09	4.80b,e ± 0.04	4.05c,f ± 0.10
	50	5.00a,d ± 0.00	5.00b,e ± 0.02	4.90b,e ± 0.05	4.30c,f ± 0.04
Edge browning	20	5.00a,d ± 0.00	4.00b,d ± 0.23	3.92b,d ± 0.12	2.85c,d ± 0.08
	30	5.00a,d ± 0.00	4.50b,d ± 0.16	3.95b,d ± 0.10	3.89c,e ± 0.05
	40	5.00a,d ± 0.00	4.50b,d ± 0.09	3.96b,d ± 0.05	3.90c,e ± 0.10
	50	5.00a,d ± 0.00	5.00b,e ± 0.03	5.00b,e ± 0.03	4.85c,f ± 0.17
Texture	20	5.00a,d ± 0.00	5.00b,d ± 0.00	4.90b,d ± 0.03	4.10c,d ± 0.03
	30	5.00a,d ± 0.00	5.00b,d ± 0.00	4.95b,d ± 0.01	4.08c,d ± 0.11
	40	5.00a,d ± 0.00	5.00b,d ± 0.00	5.00b,d ± 0.00	4.10c,d ± 0.06
	50	4.65a,e ± 0.08	4.09b,e ± 0.13	3.80b,e ± 0.14	2.22c,e ± 0.11
Color	20	5.00a,d ± 0.00	5.00b,d ± 0.00	5.00b,d ± 0.00	3.90c,d ± 0.17
	30	5.00a,d ± 0.00	5.00b,d ± 0.00	5.00b,d ± 0.00	4.02c,d ± 0.09
	40	5.00a,d ± 0.00	5.00b,d ± 0.00	5.00b,d ± 0.00	3.85c,d ± 0.10
	50	5.00a,d ± 0.00	4.23b,e ± 0.02	3.80b,e ± 0.15	2.06c,e ± 0.21
Sensory acceptability	20	5.00a,d ± 0.00	5.00b,d ± 0.00	3.80b,d ± 0.10	3.01c,d ± 0.09
	30	5.00a,d ± 0.00	5.00b,d ± 0.00	4.45b,e ± 0.09	4.05c,e ± 0.11
	40	5.00a,d ± 0.00	5.00b,d ± 0.00	4.75b,e ± 0.12	4.05c,e ± 0.13
	50	5.00a,d ± 0.00	4.04b,e ± 0.24	3.50b,d ± 0.08	2.05c,f ± 0.30

[a]5-point scale: 5 = very good and 1 = poor.
[b]Values within a same row with different letters (a,b,c) are significantly different ($P < 0.05$); values within a same column with different letters (d, e, f) are significantly different ($P < 0.05$).
[c]Mean scores of 3 independent lots. Samples in each lot were run by duplicate.

populations on lettuce leaves but would not prevent their growth during refrigerated storage. Actually, they could accelerate their growth during storage. Similar results were informed by Li and others (2001) who reported initial reductions in the populations of some microorganisms groups naturally occurring on iceberg lettuce after heat treatments at 50 °C and enhanced microbial growth during storage. Li and others (2002) reported that *Listeria monocytogenes* inoculated on cut iceberg lettuce and exposed at 50 °C for 90 s presented an accelerated growth during subsequent storage at 5 °C and 15 °C. However, heat shock at 30 °C, which did not reduce initial mesophilic counts, would trigger the growth of these populations during storage (Figure 3).

Sensory acceptability

The sensory attributes of samples subjected to heat shocks are presented in Table 1, together with those of the control samples. At 0 d, heat shock application did not affect lettuce sensory attributes, except for a little softening of lettuce exposed to 50 °C. Li and others (2001) reported similar ratings for iceberg lettuce treated with chlorinated water at 20 °C and 50 °C, although these ratings were significantly lower than those of untreated lettuce.

The different sensory indices showed different trends during refrigerated storage. Although samples treated at 50 °C presented high ratings on midrib and edge browning throughout storage, they presented the poorest ratings in texture and surface color (at the end of storage).

It is generally accepted that cut edges of stored lettuce turn brown because phenylalanine- ammonia-lyase (PAL) activity is stress-induced in the cuts and the biosynthesis of polyphenols is promoted. Mild heat shocks at 50 °C would be sufficient to affect PAL activity and this would be reflected in the stability in the ratings of midrib and edge browning in samples treated at 50 °C that remained low after 6 d of storage (Table 1). Loaiza-Velarde and others (1997) reported that treatment for 90 s at 45 °C disrupts the wound-induced increase in PAL activity, delays the accumulation of phenolic compounds, and diminishes tissue browning. Saltveit (2000) reported that treatment for 90 s at 45 °C was so persistent in fresh-cut lettuce that it did not show any browning, even after 15 d in air at 5 °C. Treatments of whole lettuce leaves at 30 °C and 40 °C also retarded browning (Table 1). Their ratings were significantly better ($P < 0.05$) than those of the control samples but not as good as those of samples treated at 50 °C (d = 6).

Samples treated at 50 °C presented the lowest ratings on color and texture. These temperature levels would be high enough to disrupt the fragile lettuce structure promoting textural changes, the liberation of intracellular compounds, and the proliferation of microorganisms. All these phenomena would cause the decay of these sensory attributes. Li and others (2001) also informed better sensory ratings for iceberg lettuce treated at 20 °C when compared with samples treated at 50 °C. They suggested that water and nutrients from tissue fluids remained on heat-treated lettuce leaves, enhancing microbial growth during storage and reducing the shelf life as a consequence of microbial decay. With control samples adversely affected by enzymatic browning and samples treated at 50 °C adversely affected by a rapid degradation of the color and texture attributes, the samples treated at 30 °C and 40 °C presented better overall sensory acceptability after 6 d of storage.

Conclusions

Heat shock treatments constitute an alternative for the preservation of organically cultivated crops when the use of synthetic chemicals is objectionable. However, its application on completely fresh lettuce leaves of the Cos variety produced results that must be critically analyzed. On 1 side, a thermal shock of 2 min at 50 °C produced significant reductions in the initial counts of mesophilic aerobic microorganisms and delayed midrib and edge browning during 6 d of storage. However, on the other side, this treatment would cause tissue damage with adverse effects on color and texture. This damage could also promote microorganism proliferation, so that after some days of storage, the benefit of the initial reduc-

tion in their numbers would be overcome. This treatment would also result in the greatest losses in ascorbic acid.

Heat shocks of 50 °C could be useful for short-term preservation of lettuce, before the onset of microbial growth, when the benefits of microbial reduction and suppression of enzymatic browning prevail and the degradation of color and texture are still acceptable.

Acknowledgments

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Univ. Nacional de Mar del Plata (UNMDP).

References

Box GEP, Hunter WG, Hunter JS. 1978. Statistics for experimenters. In: An introduction to design, data analysis and models building. New York: Wiley. 310 p.
Delaquis PJ, Fukumoto LR, Toivonen PMA, Cliff MA. 2004. Implications of wash water chlorination and temperature for the microbiological and sensory properties of fresh-cut iceberg lettuce. Postharvest Biol Technol 31(1):81–91.
Delaquis PJ, Stewart S, Toivonen PMA, Moyls AL. 1999. Effect of warm, chlorinated water on the microbial flora of shredded iceberg lettuce. Food Res Int 32:7–14.
Kader AA. 1992. Postharvest technology of horticultural crops. Calif., U.S.A.: Univ. of Calif., Div. of Agriculture and Natural Resources. 15 p.
Li Y, Brackett RE, Chen J, Beuchat LR. 2002. Mild heat treatments of lettuce enhances growth of *L. monocytogenes* during subsequent storage at 5 or 15°C. J. Appl Microbiol 92(2):269–75.
Li Y, Brackett RE, Shewfelt RL, Beuchat LR. 2001. Changes in appearance and natural microflora on iceberg lettuce treated in warm, chlorinated water and then stored at refrigeration temperature. Food Microbiol 18:299–308.
Loaiza-Velarde JG, Saltveit ME. 2001. Heat shock applied either before or after wounding reduces browning of lettuce leaf tissue. J Am Soc Hortic Sci 126:227–34.
Loaiza-Velarde JG, Tomás-Barberá FA, Saltveit ME. 1997. Effect of intensity and duration of heat shock treatments on wound-induced phenolic metabolism in iceberg lettuce. J Am Soc Hortic Sci 122:73–7.
Logomarsino JV, Gao J. 1991. Ascorbic acid retention of broccoli held on a refrigeration salad bar. J Food Qual 14:513–9.
López-Gálvez G, Saltveit ME, Cantwell MI. 1996. Wound-induced phenylalanine ammonia lyase activity: factors affecting its induction and correlation with the quality of minimally processed lettuce. Postharvest Biol Technol 8:179–90.
Moreira MR, Roura SI, del Valle CE. 2003. Quality of Swiss chard produced by conventional and organic methods. Lebensm Wiss Technol 36:135–41.
Murata M, Tanaka E, Minoura E, Homma S. 2004. Quality of cut lettuce treated by heat shock: prevention of enzymatic browning, repression of phenylalanine ammonia-lyase activity and improvement on sensory evaluation during storage. Biosci Biotechnol Biochem 68:501–7.
Pelletier O. 1985. Vitamin C: L-ascorbic and dehydro-L-ascorbic acids. In: Augustin J, Klein BP, Becker D, editors. Methods of vitamin assay. 4th ed. New York: John Wiley & Sons. p 125–32.
Reyes V. 1996. Improved preservation systems for minimally processed vegetables. Food Australia 48(2):87–90.
Roura SI, Davidovich LA, del Valle CE. 2000. Postharvest changes in fresh Swiss chard under different storage conditions. J Food Qual 23:143–47.
Roura SI, Moreira MR, Ponce AG, del Valle CE. 2003. Dip treatments for fresh Romaine lettuce. Ital J Food Sci 3(15):405–15.
Saltveit ME. 1998. Heat shock and fresh cut lettuce. Perishables Handling Q 95:5–6.
Saltveit ME. 2000. Wound induced changes in phenolic metabolism and tissue browning are altered by heat shock. Postharvest Biol Technol 21(1):61–9.
Skandamis P, Koutsoumanis K, Fasseas K, Nychas GJE. 2001. Inhibition of oregano essential oil and EDTA on *Escherichia coli* O157:H7. Ital J Food Sci 13:55–65.
Vamos-Vigyazo L. 1981. Polyphenol oxidase and peroxidase in fruits and vegetables. Crit Rev Food Sci Nutr 15:49–127.
Volk W. 1980. Applied statistics for engineers, correlation-regression. New York: McGraw-Hill, Inc. 260 p.
Wadsö L, Gomez F, Sjöholm I, Rocculi P. 2004. Effect of tissue wounding on the results from colorimetric measurements of vegetable respiration. Thermochim Acta 422(1–2):89–93.

URLs and E-mail addresses are active links at www.ift.org

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Postharvest Biology and Technology 31 (2004) 81–91
Postharvest Biology and Technology
www.elsevier.com/locate/postharvbio

Implications of wash water chlorination and temperature for the microbiological and sensory properties of fresh-cut iceberg lettuce☆

Pascal J. Delaquis*, Lana R. Fukumoto, Peter M.A. Toivonen, Margaret A. Cliff

Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, 4200 Highway 97, Summerland, BC, Canada V0H 1Z0

Received 18 July 2002; accepted 26 April 2003

Abstract

Cut iceberg lettuce (*Lactuca sativa* L.) was washed in chlorinated water (0–100 mg l^{-1} total chlorine) at temperatures up to 54 °C to identify a practical commercial process. Based on relative leakage rates and visual assessment of the packaged, stored lettuce, a 1 min treatment at 50 °C yielded acceptable quality. Comparison with cut lettuce washed at 4 °C revealed that disinfection of the lettuce was improved by heat, although the difference in total microbial populations was only 1 log cfu g^{-1}. Chlorine addition up to 100 mg l^{-1} did not appreciably increase removal of micro-organisms from the lettuce surface at either temperature, although micro-organisms were eliminated from wash water at the lowest concentration applied (25 mg l^{-1} total chlorine). Chlorine losses were minimal in wash solutions adjusted to pH 6.1 but exceeded 50% at 50 °C, and this parameter was not adjusted for experiments on the sensory quality of lettuce washed at both temperatures. The aromas of lettuce washed in tap water at 4 or 50 °C for 1 min were not significantly different ($P > 0.05$) after 1 or 7 days of storage at 1 °C. Addition of chlorine (100 mg l^{-1} total) significantly reduced ($P < 0.05$) aroma development during storage for 1 or 7 days following a wash at 50 °C, but not at 4 °C. Sensory comparison of lettuce washed at 50 °C with 0, 10, 25, 50, 75 and 100 mg l^{-1} total chlorine by an *R*-index test procedure indicated that differences in aroma were more apparent at chlorine concentrations >50 mg l^{-1}.
© 2003 Published by Elsevier B.V.

Keywords: Wash water; Iceberg lettuce; Chlorine; Sensory quality; Microbiology

1. Introduction

Unit operations applied in fresh-cut lettuce processing usually include trimming, core removal, cutting or slicing, washing, drying and packaging (King et al., 1991). Washing ensures the elimination of soil and plant debris, thereby improving the appearance of the product. In conventional processing schemes, this operation also reduces product temperature, ostensibly to limit development of physiological disorders and microbial proliferation during subsequent storage (Francis and O'Beirne, 1997; Simons and Sanguansri, 1997). Chilled, chlorinated water is commonly used for this purpose, although alternative washing systems that employ ozone, peroxyacetic acid or hydrogen

☆ PARC paper contribution no. 2173.

* Corresponding author. Tel.: +1-250-494-6367; fax: +1-250-494-0755.
E-mail address: delaquisp@agr.gc.ca (P.J. Delaquis).

0925-5214/$ – see front matter © 2003 Published by Elsevier B.V.
doi:10.1016/S0925-5214(03)00134-0

peroxide are gradually gaining acceptance (Mermelstein, 1998).

Appropriate washing systems must ensure the removal or destruction of micro-organisms in water. The USFDA recommends 50–200 mg l^{-1} total chlorine at pH 6.0–7.5 and contact times of 1–2 min for this purpose (USFDA, 1998). The International Fresh-Cut Produce Association (IFPA) Model HACCP Plan for shredded lettuce, suggests a maximum chlorination of 100–150 mg l^{-1} total chlorine at pH 6.0–7.0 and the maintenance of 2–7 mg l^{-1} free residual chlorine after contact (Flickinger, 1999). An understanding of chlorine chemistry and a definition of terms are required to interpret these guidelines. Total chlorine refers to the sum of free and combined chlorine in solution (Food Processors Institute, 1980). Free chlorine, free available and free residual chlorine refer to chlorine in the form of elemental chlorine (Cl_2), hypochlorous acid (HOCl) and hypochlorous ion (OCl^-) (Simons and Sanguansri, 1997). Hypochlorous acid, the more active antimicrobial form, reacts quickly with inorganic and organic matter in solution to form combined chlorine compounds that have limited activity. The amount of HOCl required to react with such material is defined as the chlorine demand of the water. Excess HOCl beyond the chlorine demand is referred to as free residual chlorine. Free residual chlorine concentrations between 2 and 7 mg kg^{-1} are required to ensure complete destruction of micro-organisms in water (Food Processors Institute, 1980). The chemical state of chlorine is affected by pH and temperature. At pH 3–6, almost all chlorine is present in the form of HOCl, while Cl_2 predominates as pH decreases below 3.0 and OCl^- and H^+ predominates at pH >7.0. A pH range of 6.0–7.5 is therefore often recommended to maintain high levels of HOCl and to minimize equipment corrosion (Food Processors Institute, 1980).

Chlorinated water is believed to provide some disinfection of the lettuce surface, although several reports suggest that the effect is minimal at best (Adams et al., 1989; Beuchat and Brackett, 1990; Beuchat, 1992; Brackett, 1987; Delaquis et al., 1999). The effectiveness of chlorinated water washes against micro-organisms on the surface of cut lettuce can be improved by increasing process temperatures. Delaquis et al. (1999, 2000) showed that residual microbial populations were 2 log cfu g^{-1} lower on cut lettuce washed in chlorinated (100 mg l^{-1}) water at 47 °C for 3 min compared with product washed at 4 °C. In addition, browning of the packaged, stored lettuce is delayed by heat-induced suppression of phenylalanine ammonia lyase (PAL) activity. The time of exposure required to achieve this effect is critical since sufficient heat is required to inactivate PAL without causing injury to the tissues (Fukumoto et al., 2002; Loaiza-Verlarde et al., 1997). While the advantages derived from such a process are explicit, the relatively long contact time required to achieve control of browning would hamper commercial application of the process. Line speeds in most plants generally provide contact times of 60 s or less. Equivalent treatments that are effective within this time frame are therefore needed to permit large scale production of lettuce in flumes operated at high temperatures. In addition, little is known about the effect of chlorine on the sensory properties of lettuce. Excessive free chlorine concentrations ($\geq$200 mg l^{-1}) have been reported to cause adverse discolorations and the appearance of off-flavours (Hurst and Schuler, 1992). Sensory evaluation of iceberg lettuce washed at 47 °C revealed that an atypical aroma developed during storage (Delaquis et al., 2000). It remains unclear whether such defects are derived from chlorine induced physiological changes or from the formation of volatile chlorinated compounds.

The main objective of this study was to determine the influence of wash water temperature and chlorine concentration on the storage quality, microbiological characteristics and sensory properties of cut iceberg lettuce. A secondary objective was the identification of processing parameters appropriate for commercial production of cut lettuce at high temperatures.

2. Materials and methods

2.1. Lettuce preparation

Heads of iceberg lettuce (*Lactuca sativa* L., cv. Salinas) grown in the USA (AZ or CA) or Canada (BC), depending on availability, were obtained from a local retailer. Wrapper leaves were removed by hand and cores were excised with a stainless steel tube sharpened at one end. The heads were then cut into quarters from top to stem using a sharp stainless steel knife. Lettuce used for storage studies and sensory analysis

was prepared by cutting the quarters crosswise into ~4 cm^2 pieces with a slicer (Model 410, Hobart Manufacturing Co., Troy, OH, USA). Lettuce used for microbiological studies was prepared in similar fashion but core leaves from each quarter (about half of each quarter) were removed before cutting. This procedure reduced sampling variations induced by low microbial populations in inner leaves.

2.2. Effect of chlorinated water temperature on storage quality and tissue damage

Samples (50 g) of cut lettuce were placed in a stainless steel mesh basket. The basket was lowered into 14 l chlorinated water (100 mg l^{-1} total chlorine, pH 8.7) in a water bath equipped with a circulating heater. Commercial grade NaOCl (6%, w/v) was used to prepare chlorine solutions. The following time/temperature conditions were applied: 4 °C for 1, 2, 3, 4 and 5 min; 47 °C for 1, 2, 3, 4 and 5 min; 48 °C for 1, 2, 3, 4 and 5 min; 49 °C for 1, 2, 3, 4 and 5 min; 50 °C for 1, 2, 3, 4 and 5 min; 51 °C for 0.5, 1, 1.5, 2 and 2.5 min; 52 °C for 0.5, 1, 1.5, 2 and 2.5 min; 53 °C for 0.5, 1, 1.5, 2 and 2.5 min; and 54 °C for 0.5, 1, 1.5, 2 and 2.5 min. Experiments at 4 °C were carried out with pre-chilled water in a cold room set at that temperature. The lettuce was slowly agitated with a spoon to ensure uniform heating of the pieces. At the end of treatment, the basket was immediately placed in a pail filled with tap water (~10 °C) for 1 min, and the lettuce was spun in a home salad spinner to remove excess water. Samples of photosynthetic (green) tissue were removed for relative leakage rate (RLR) measurements. The remainder of the lettuce was packed into PD941 bags (17 cm × 18 cm; Cryovac Corp., Mississauga, ON, Canada; oxygen transmission rate: 77.8 pmol s^{-1} m^2 Pa^{-1}). The bags were sealed using a SwissVac MINOR 2 vacuum sealer (Luzern, Switzerland) and stored flat on metal mesh shelves, in a single layer, at 5 ± 1 °C in the dark. The lettuce was visually inspected for browning and tissue damage during up to 14 days of storage using the five-point scale of Peiser et al. (1998), where 1 = none, 3 = moderate and 5 = severe. Three replicate trials were conducted at each temperature.

Relative leakage rates were determined from measurements on eight pieces of photosynthetic tissue. Five strips (2 mm in width) were excised simultaneously from each piece with a tool fashioned from six utility knife blades. The strips were cut into ~1.5 cm lengths, 40 of which were collected into 50 ml Erlenmeyer flasks. Deionized distilled water (25 ml) was added to the flasks. After 20 s of manual swirling, the rinse was discarded, another 25 ml deionized water was added and the flasks were placed on an orbital shaker (Lab-Line Instruments Inc., Melrose Park, IL, USA) operated at 1500 min^{-1} for 2 h at ~22 °C. Deionized distilled water (1 ml) was then added to each flask and a 1 ml sample was removed for measurement of absorbance as described further. The flasks were placed in a freezer at −25 °C for at least 2.5 h, and thawed at room temperature. Deionized distilled water (1 ml) was added to each flask and 1 ml samples were removed. Absorbance of the samples was measured at 280 nm using a spectrophotometer. The RLR was calculated using the formula (Toivonen, 1992):

$$\mathrm{RLR} = \frac{A_{280}\ \text{before freezing}}{A_{280}\ \text{after freezing}} \times 100$$

2.3. Changes in free and total chlorine concentrations in wash water at 4 or 50 °C

Tap water (4 l) chilled to 4 °C or heated to 50 °C was continuously mixed with a Haake circulating immersion heater (Model 002-4175, 1 W, Germany) in a stainless steel water bath. Sufficient commercial grade NaOCl (6%, w/v) was added to obtain a 100 mg l^{-1} total chlorine solution (~98 mg l^{-1} free chlorine) and pH was adjusted to 6.1 with 2% citric acid, where required. Samples were withdrawn every 5 min for 100 min after addition of the NaOCl. Free and total chlorine concentrations were measured at each sampling interval with a commercial test kit (Model CN-66, Hach, Loveland, CO, USA), and pH was measured with a pH meter. Three trials were conducted for each combination of temperature and pH.

2.4. Effect of temperature and chlorine concentrations on the antimicrobial efficacy of chlorinated water

Samples (150 g) of cut lettuce were washed for 1 min with constant stirring in 4 l water at 4 or 50 °C with 0, 25, 50, 75 and 100 mg l^{-1} total chlorine. The

lettuce was spun in a home lettuce spinner after treatment. Two sub-samples (50 g) from each treatment were immediately blended with 450 ml of 0.1% peptone in a Lab Stomacher (Colworth, UK). Duplicate aliquots (0.1 ml) of appropriate dilutions were surface spread onto plate count agar (Difco, Becton Dickinson and Co., Sparks, MD, USA) which was incubated at 30 °C for 48 h for estimation of total microbial populations. Three replicate trials were performed. Duplicate water samples (1 ml) were collected after washing and were subjected to identical microbiological analyses to determine populations of micro-organisms in the wash water.

storage and held at room temperature for 1 h. Approximately, 70 g were placed into 36–450 ml translucent plastic cups (Dixie brand, Fort James Canada Inc., Toronto, Ont., Canada). The cups were covered with a disposable Petri dish lid, and each was labelled with a three-digit random number. Two triangle tests, consisting of three samples were prepared for 12 panelists. Each set included one odd sample. All panelists assessed the same sample sets.

2.5.1.3. Triangle test procedures. Two triangle tests were performed on three different mornings according to the given schedule.

Test day	Test 1			Test 2		
	Temperature (°C)	[Cl] (mg l^{-1})	Day	Temperature (°C)	[Cl] (mg l^{-1})	Day
1	4	0	1	50	0	1
1	4	0	7	50	0	7
2	4	0	1	4	100	1
2	50	0	1	50	100	1
3	4	0	7	4	100	7
3	50	0	7	50	100	7

2.5. Sensory quality of cut lettuce washed in chlorinated water

2.5.1. Comparison of lettuce washed at different temperatures by triangle testing

2.5.1.1. Lettuce processing. Samples (5.5 kg) of cut iceberg lettuce were prepared as described earlier. Each sample was placed in a stainless steel basket, which was lowered into 80 l wash water in a tank built for this purpose (Delaquis et al., 1999). The lettuce was washed for 1 min under the following conditions: 4 or 50 °C without chlorine, and 4 or 50 °C water with 100 mg l^{-1} total chlorine (pH 8.7). Excess water was removed in a commercial centrifuge (Model FP35, Bock Engineered Products Inc., Toledo, OH, USA) operated at 600 min^{-1} for 2 min and the lettuce was packed into five PD941 bags (35 cm × 55 cm) which were sealed as before. All bags were stored in a single layer on metal mesh shelves at 1 ± 1 °C in the dark until analyzed.

2.5.1.2. Sample preparation. Five bags of lettuce from each treatment were removed from refrigerated

Twenty-four untrained panelists (14 females and 10 males) from the staff at the Pacific Agri-Food Research Centre (PARC) were recruited for the analyses. Panelists were familiarized with the use of the triangle test prior to the first session. Analyses were conducted in individual tasting booths under red lights. Panelists were given two triangle tests for each comparison and were asked to smell the samples in the order given. They were asked to identify the odd sample and to provide written descriptors on the nature of the difference, where possible. After a short break, the same procedure was applied for the comparison of the remaining sets of samples. The presentation order of the comparisons and samples were all completely randomized.

2.5.1.4. Statistical analyses. The number of correct responses for each triangle test (24 panelists, 2 replicates) were tabulated. The values were adjusted, using an over dispersion value, to account for replication in discrimination tests as recommended by Brockhoff and Schlich (1998). The probability of correct responses for triangle tests was determined from the table in Poste et al. (1991).

2.5.2. Comparison of lettuce washed at 50 °C with different levels of chlorine by R-index testing

2.5.2.1. Lettuce processing. Cut lettuce was divided into twelve 1.050 kg lots. Seven were washed for 1 min in a water bath containing 15.8 l unchlorinated water at 50 °C. The remaining lots were divided into two 525 g samples which were washed separately in water containing 10, 25, 50, 75 or 100 mg l^{-1} total chlorine, without pH adjustment, also at 50 °C. The washed lettuce was dried and packaged as before and the bags were stored for 1 day at 1 ± 1 °C in the dark.

2.5.2.2. Sample preparation. All samples were removed from refrigerated storage and held at room temperature for 1 h prior to analysis. Lettuce (approximately 70 g) washed in unchlorinated water was dispensed into 72 translucent plastic cups (450 ml) which were covered with Petri dish lids. These cups were labelled with the code "R". Lettuce from the other treatments was likewise dispensed into 12 cups labelled with random three-digit codes. Six "same–different" assortments of two samples (R and each treatment) were prepared for each panel.

2.5.2.3. Sensory methods. Sensory evaluation was conducted at a large table, partitioned with white wooden dividers to create individual tasting stations. Each station was assembled with six "same–different" assessments, consisting of the reference (R) and coded samples. Thirty-six panelists (16 female, 20 male, PARC employees) were recruited to participate in the assessment. After a brief training session to acquaint the panelists with the methodology they were asked to: (1) evaluate the pairs in the order given; (2) determine whether the coded sample was the 'same' or 'different' from the R sample; (3) indicate their level of confidence with their response as 'sure' or 'unsure'; and (4) describe the nature of the difference, if possible. The presentation order of the pairs was completely randomized. Data were tabulated as the frequency of response in each of the four categories (different–sure, different–unsure, same–unsure, same–sure).

2.5.2.4. Statistical analysis. Lettuce samples wabreak shed at 50 °C for 1 min in water with different amounts of chlorine (0, 10, 25, 50, 75 or 100 mg l^{-1} total chlorine) were compared pairwise with lettuce washed in unchlorinated water (reference R) after 1 day of storage at 1 °C. An *R*-index response matrix was constructed from *R*-index values calculated using the following equation (O'Mahony, 1992):

$$R_i = \frac{a_i(f+g+h) + b_i(g+h) + c_i(h) + 0.5(a_i e + b_i f + c_i g + d_i h)}{(a_i + b_i + c_i + d_i)(e+f+g+h)} \quad (1)$$

	Different–sure	Different–unsure	Same–unsure	Same–sure
Signal$_i$ (sample)	a_i	b_i	c_i	d_i
Noise (reference)	e	f	g	h

where the signal values (a_i–d_i) represent the frequency of responses for R compared with the treatments, and noise values (e–h) are the frequency responses for R samples compared with another R sample. The R_i index takes on values between 0.5 and 1.0 and represents the proportion of the population that can detect a difference between the treated and untreated samples. Critical values for determining the significance of the R_i index were identified using one-tailed values provided by Bi and O'Mahony (1995).

3. Results and discussion

3.1. Storage quality and tissue damage

The quality of packaged, cut iceberg lettuce was assessed after washing in chlorinated water at various temperatures. Treatments resulting in immediate evidence of tissue damage, particularly translucence, softening and exudation, were discontinued. The remaining treatments led to variable effects on the onset of browning in the stored samples (Table 1). Moderate browning was evident in unwashed lettuce and in lettuce washed at 4 °C after 4 days. In contrast, heat treatments delayed the onset of browning at all exposure times. There was no evidence of browning in cut lettuce heated at 47 °C for 180 s after 9 days in storage, a

Table 1
Time required for the appearance of moderate browning in cut lettuce stored at 5 °C following treatment in 100 mg l^{-1} chlorine at various temperatures and exposure times

Temperature (°C)	Time (s)	Days[a] until moderate browning[b]
Unwashed lettuce		4.0 ± 0
4	60	4.0 ± 0
4	120	4.0 ± 0
4	180	4.0 ± 0
4	240	4.0 ± 0
4	300	4.0 ± 0
47	60	7.0 ± 0
47	120	9.0 ± 0
47	180	>9
47	180, no cooling	>9
48	60	7.7 ± 1.2
48	120	>9
48	180	>9
49	60	9.3 ± 0.6
49	120	>9
49	180	>9
50	60	>9
50	60, no cooling	>9
51	30	7.7 ± 1.2
51	60	>9

Where indicated, samples were not cooled in tap water prior to packaging.

[a] Each value represents the mean of three replicates followed by the standard deviation.

[b] Moderate browning is defined by Peiser et al. (1998) as the midpoint on a five-point scale from none to severe.

result in agreement with our previous report (Delaquis et al., 1999). Relative leakage rates (Fig. 1) provided estimates of tissue damage induced by the treatments. Photosynthetic tissue was used for the measurement because variability in the thickness of vascular (rib) tissue led to inconsistent results (data not shown). The RLR of unwashed iceberg lettuce tissue was 21 ± 3%, and measurements derived from lettuce treated at 4 °C for 5 min remained within this range for all exposure times. Heat treatments at temperatures <50 °C for 60 s also maintained the RLR near this value. Higher temperatures and prolonged exposures led to the appearance of tissue damage, particularly when RLR values exceeded approximately 30%.

Suppression of browning was achieved with the following treatments, which could serve as alternatives to a 180 s wash at 47 °C: 120–180 s at 48 °C, 120 s at 49 °C, 60 s at 50 °C and <60 s at 51 °C. Since process times required at 48 and 49 °C are greater than desired for a practical commercial process, only treatments at 50 or 51 °C are suitable alternatives. However, RLR values for these treatments approach the point where tissue damage becomes evident (Fig. 1). The consequences of excessive heating at these temperatures was demonstrated by omission of a cooling step. Tissue damage manifested by a translucent appearance and exudation in the package was evident in the stored product. Process control therefore becomes critical with the brief exposure times required at temperatures >50 °C. For these reasons, a 60 s exposure at 50 °C offers the best alternative to treatment at 47 °C and these process parameters were used for the remainder of the study. It should be stressed that the raw materials used in these investigations were of good quality and there was little variability in RLR indices prior to processing. The quality of raw produce is variable however, and the extent to which this would affect the susceptibility of iceberg lettuce tissues to heat induced damage is unknown. Clearly, the relationship between physiological status of lettuce tissues and susceptibility to heat treatment must be examined in the future.

3.2. Fate of chlorine in wash water at 4 or 50 °C

Changes in water pH and chlorine concentrations in sodium hypochlorite solutions held for 100 min at 4 or 50 °C are shown in Fig. 2. Free chlorine concentrations were 1–3 mg kg^{-1} lower than total chlorine in all samples (data not shown), indicative of the low chlorine demand of the tap water used for these experiments. Chlorine demand of drinking water is normally between 0.25 and 1.00 mg l^{-1} (Mercer and Somers, 1957). Comparatively higher values noted in this study were likely the result of compound errors associated with the accuracy of the test kit and the need for dilution of the samples prior to analysis. Addition of NaOCl to water was expected to raise pH due to dissociation of the molecule to HOCl and NaOH. At room temperature, the pH of an unbuffered 100 mg l^{-1} total chlorine solution was 8.7, a value similar to that reported by Adams et al. (1989) (pH 8.8) and Escudero et al. (1999) (pH 8.5). The pH of NaOCl solutions changed slightly over time however, and the rate was slightly affected by temperature (Fig. 2b).

Loss of chlorine from solution was dependant on pH and temperature (Fig. 2a). The molecular state of

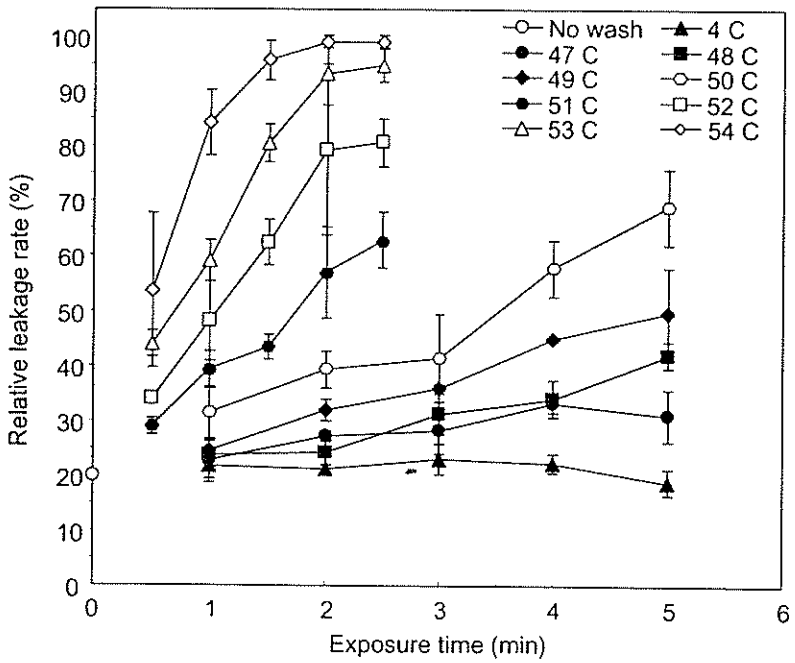


Fig. 1. Relative leakage rates of lettuce photosynthetic tissues exposed to water containing 100 mg l^{-1} total chlorine at various temperatures and times. Values are means of three replicates and bars represent one standard deviation.

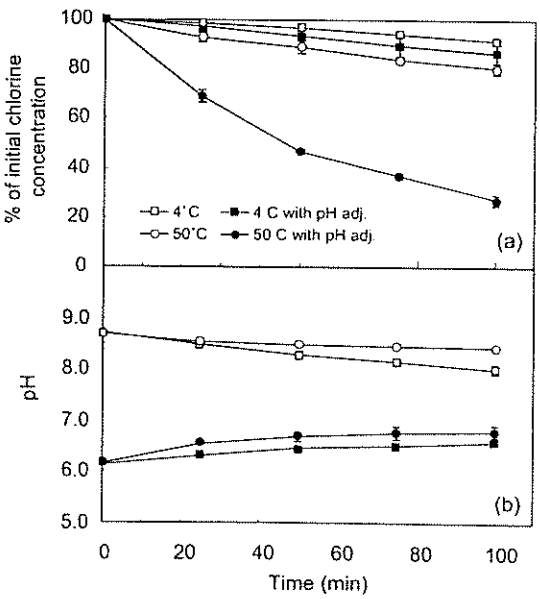


Fig. 2. Change in total chlorine (a) and pH (b) of recirculated tap water containing 100 mg l^{-1} total chlorine at 4 or 50 °C with and without pH adjustment to 6.1.

NaOCl is influenced by pH and the formation of elemental chlorine from HOCl is favoured as pH decreases (Food Processors Institute, 1980). Evaporation of chlorine is also accelerated by heat, although there is a paucity of data for temperatures above ambient. These factors were likely responsible for the rapid loss of chlorine from solutions at 50 °C adjusted to pH 6.1. Acidification of chlorine solutions to pH 6.0–7.5 to maintain higher levels of HOCl and to enhance antimicrobial effectiveness is recommended by the USFDA (1998). Accelerated loss of chlorine at 50 °C would preclude acidification since this would require excessive additions of NaOCl during processing to maintain desired free chlorine levels. The pH of chlorine solutions was therefore not adjusted for the remainder of this study.

3.3. Antimicrobial efficacy of chlorinated water

The effects of chlorine concentration and wash water temperature on the native microflora of cut iceberg lettuce are shown in Fig. 3. Unchlorinated water at 4 °C reduced total microbial populations

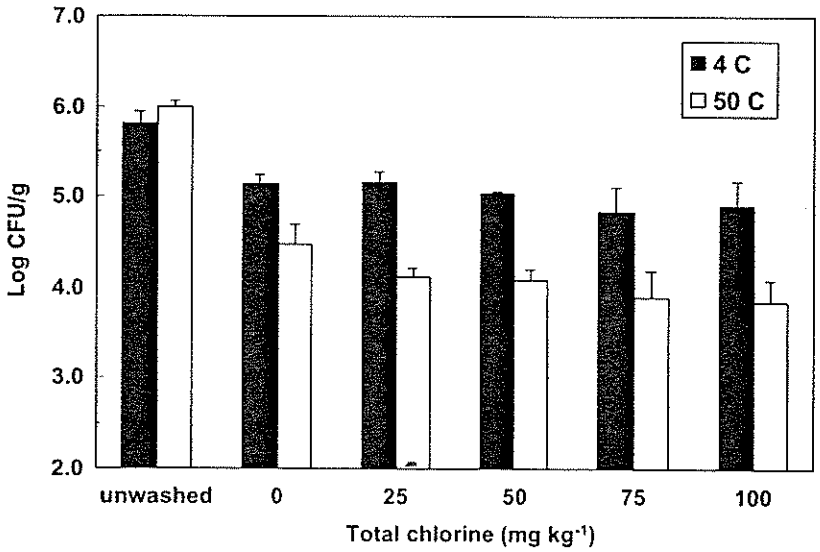


Fig. 3. Microbial populations on lettuce after washing at 4 or 50 °C for 1 min with different concentrations of total chlorine. Values are averages of three replicates and bars represent one standard deviation.

by <1 log cfu g^{-1}. Population reductions remained <1 log cfu g^{-1} at the highest chlorination level. Washing in unchlorinated water at 50 °C led to reductions of approximately 1.5 log cfu g^{-1}. The microflora of iceberg lettuce is dominated by Gram-negative bacteria, particularly species of *Pseudomonas* (56.7%), *Serratia* (8.1%) and *Erwinia* (8.1%) (King et al., 1991), micro-organisms with little resistance to heat. Exposure to a temperature of 50 °C was probably sufficient to inactivate some of the more heat sensitive species. The effect was slightly enhanced by chlorine and total populations were reduced by an average of 2 log cfu g^{-1}. Hence, chlorination of wash water without pH adjustment at either temperature failed to appreciably enhance the removal or destruction of micro-organisms from the cut lettuce surface.

These results are in agreement with previous work by Adams et al. (1989) who observed <1 log cfu g^{-1} reductions on English Webb lettuce washed in cold water with 0–300 mg l^{-1} free chlorine without pH adjustment. Investigations carried out with inoculated lettuce have occasionally led to different conclusions. Behrsing et al. (2000) reported reductions in *Escherichia coli* populations between 1.9 and 2.8 log cfu g^{-1} after 30 s contact with 50 or 100 mg l^{-1} free chlorine solutions adjusted to pH 6.0–6.5, compared to 1.5–1.8 log cfu g^{-1} with unchlorinated water. Escudero et al. (1999) observed 1–2 log cfu g^{-1} reductions in *Yersinia enterocolitica* populations after washing with 25–300 mg l^{-1} total chlorine without pH adjustment. In contrast, Weissinger et al. (2000) observed <1 log cfu g^{-1} reductions in populations of *Salmonella* baildon washed in 120 or 200 mg l^{-1} free chlorine (adjusted to pH 6.8 in phosphate buffer) for 40 s, and Li et al. (2001) found that 20 mg l^{-1} free chlorine (pH 7.0, unadjusted) did not substantially decrease *E. coli* O157:H7 populations after 90 s at 20 or 50 °C. Differences in the design of these studies, particularly with respect to the nature of the inoculum and inoculation procedures, could account for disparities in observations. The inability to remove the bulk of the native microflora by washing in water confirms that most native micro-organisms on lettuce are firmly bound to the surface. Time elapsed between inoculation and application of a wash would therefore influence the efficacy of treatments, because individual bacterial strains differ in their ability to attach and surface colonization is time dependent. The limited wettability of aqueous chlorine solutions would preclude removal cells attached to the lettuce surface.

Water from one of the replicate trials was analyzed immediately after washing to determine the fate of micro-organisms removed from lettuce. No micro-organisms were detected (limit of detection = 10 cfu ml^{-1}) when wash water was supplemented with 25, 50, 75 and 100 mg l^{-1} chlorine. In contrast, 3.8 log cfu ml^{-1} viable cells were recovered from unchlorinated water after washing at 4 °C, and 1.2 log cfu ml^{-1} at 50 °C. The latter result illustrates the lethality of water at this temperature toward micro-organisms removed from the lettuce surface. Notwithstanding the effects of temperature, it is clear that free chlorine destroyed most of the micro-organisms removed from the cut lettuce surface. Minimum free chlorine levels between 2 and 7 mg l^{-1} have been recommended to ensure the microbiological quality of water used for washing vegetables (Flickinger. 1999). This is substantiated by a considerable body of data on the effect of chlorine toward planktonic bacterial cells. For example, El-Kest and Marth (1988a,b) showed that free chlorine levels between 1 and 4 mg l^{-1} inactivate *Listeria monocytogenes* within 30 s in solutions adjusted to pH 7.0 in phosphate buffer. The objective in water chlorination for washing lettuce should therefore be the maintenance of free chlorine levels within this range, irrespective of process temperature.

3.4. Sensory evaluation of lettuce washed in chlorinated water

Table 2 summarizes the comparisons of wash treatments on lettuce aroma using triangle tests. Results were not affected by odd sample presentation order, replication of tests, replication by a panelist, order of comparison, or reuse of samples (data not shown). The aromas of lettuce washed in unchlorinated water at 4 or 50 °C were not significantly different ($P > 0.05$) from each other after 1 or 7 days of storage. The temperature of the wash water therefore did not significantly affect the aroma of lettuce. However, scores recorded for lettuce stored for 7 days indicate that some panelists were able to detect differences (Table 2). It is speculated that this trend reflects variability in storage stability caused by the wash treatments.

Chlorine (100 mg l^{-1} total) in the wash water significantly ($P < 0.05$) affected the aroma of lettuce washed at 50 °C after both 1 and 7 days of storage, but not at 4 °C (Table 2). Many panelists commented that lettuce washed with 100 mg l^{-1} chlorine lacked aroma or had a 'fresher', 'cleaner' aroma than lettuce washed in tap water. Lettuce washed without chlorine was more odoriferous and was most frequently described as 'earthy' and 'musty'. These comments were noted for samples washed both at 4 or 50 °C and

Table 2
Effect of wash treatments on the aroma of iceberg lettuce compared using triangle tests (24 panelists, 2 replicates)

Comparison for triangle test	Calculated number of correct responses[a]	Calculated number of total responses[a]	Correct (%)	Probability of obtaining number of correct responses by chance[b]
Lettuce washed at 4 and 50 °C with 0 mg l^{-1} chlorine after 1 day of storage at 1 °C	15	48	31.3	0.672
Lettuce washed at 4 and 50 °C with 0 mg l^{-1} chlorine after 7 days of storage at 1 °C	21	48	43.8	0.086
Lettuce washed with 0 and 100 mg l^{-1} chlorine at 4 °C after 1 day of storage at 1 °C	17	39	43.6	0.118
Lettuce washed with 0 and 100 mg l^{-1} chlorine at 50 °C after 1 day of storage at 1 °C	25	48	52.1	0.006
Lettuce washed with 0 and 100 mg l^{-1} chlorine at 4 °C after 7 days of storage at 1 °C	12	33	36.4	0.419
Lettuce washed with 0 and 100 mg l^{-1} chlorine at 50 °C after 7 days of storage at 1 °C	17	34	50.0	0.033

[a] Values were adjusted for replication in discrimination testing as outlined by Brockhoff and Schlich (1998).
[b] From Statistical Chart 2 in Poste et al. (1991).

are consistent with those reported by Delaquis et al. (2000) for lettuce washed at 47 °C with 100 mg l^{-1} total chlorine, which lacked 'typical lettuce aroma'. It was postulated that chlorine solutions applied at higher temperatures 'bleach' or otherwise suppress odour volatiles in packaged lettuce.

Unlike the study of Delaquis et al. (2000), panelists did not comment on chlorinaceous off-odours in warm water washed lettuce. Washing treatments applied in this study (50 °C for 1 min) and previous work (Delaquis et al., 2000) (47 °C for 3 min) resulted in similar physiological changes, as shown in Fig. 2. It is speculated that panelists in the Delaquis et al. (2000) study may have experienced an expectation error (Poste et al., 1991) and may have had difficulty identifying off-odour, due to the lack of a reference sample. In this study, panelists were selected with no knowledge of the experimental treatments and were given no detailed information on the experimental treatments. The lack of comments provided by the panelists reflected the difficulty in describing an already 'neutral' product, but one which became more 'neutral' with the use of chlorine.

Since significant differences in aroma were induced by washing at 50 °C in chlorinated water, comparisons of different chlorine levels with tap water were also conducted using *R*-index tests (Table 3). Although the *R*-index values were not significantly different at $P < 0.05$, the values did increase with increasing concentration of chlorine. Many panelists described the differences in terms of intensity of typical lettuce aroma, similar to observations noted for the triangle tests. No comments on off-odours or chlorine were received. Washing in 50 mg l^{-1} total chlorine would likely eliminate changes in lettuce aroma. The change in aroma is probably not a major concern for lettuce quality though, since lettuce is usually used in salads that are covered with dressings that mask aroma, and because lettuce has very little aroma to begin with.

Table 3
R-index for aroma of iceberg lettuce washed at 50 °C with 0, 10, 25, 50, 75 or 100 mg l^{-1} total chlorine compared to reference (unchlorinated water) using 36 panelists

Concentration of total chlorine in wash (mg l^{-1})	*R*-index[a]	Probability[b] of *R*-index
0	0.500	
10	0.478	>0.2
25	0.505	>0.2
50	0.510	>0.2
75	0.628	0.05–0.10
100	0.579	0.10–0.20

[a] Proportion of individuals who detected a difference between the treated and reference samples (0 mg l^{-1} chlorine).
[b] Values obtained from the table of Bi and O'Mahony (1995).

4. Conclusions

A 1 min treatment in chlorinated water at 50 °C was shown to be appropriate for the retention of quality in packaged, cut lettuce. Sensory analysis indicated that lower chlorine levels in both cold or warm wash water would reduce lettuce aroma changes without adversely affecting the antimicrobial efficacy of the process. Lower chlorine levels would also lessen processing costs and problems associated with disposal of waste chlorinated water. Lower chlorine levels may also be desirable to reduce health hazards due to the potential toxicity, carcinogenicity and mutagenicity of chlorinated water and chloroorganic compounds formed by reaction with food components (Wei et al., 1985). These benefits would complement the improved control of browning achieved by washing in water at 50 °C.

Acknowledgements

The authors sincerely thank Kareen Stanich for her technical help and Marj King for her suggestions.

References

Adams, M.R., Hartley, A.D., Cox, L.J., 1989. Factors affecting the efficacy of washing procedures used in the production of prepared salads. Food Microbiol. 6, 69–77.

Behrsing, J., Winkler, S., Franz, P., Premier, R., 2000. Efficacy of chlorine for inactivation of *Escherichia coli* on vegetables. Postharvest Biol. Technol. 19, 187–192.

Beuchat, L.R., 1992. Surface disinfection of raw produce. Dairy Food Environ. Sanit. 12, 6–9.

Beuchat, L.R., Brackett, R.E., 1990. Survival and growth of *Listeria monocytogenes* on lettuce as influenced by shredding, chlorine treatment, modified atmosphere packaging and temperature. J. Food Sci. 55, 755–758, 870.

Bi, J., O'Mahony, M., 1995. Table for testing the significance of the *R*-index. J. Sens. Stud. 10, 431–437.

Brackett, R.E., 1987. Antimicrobial effect of chlorine on *Listeria monocytogenes*. J. Food Prot. 52, 999–1003.

Brockhoff, P.B., Schlich, P., 1998. Handling replications in discrimination tests. Food Qual. Pref. 9, 303–312.

Delaquis, P.J., Stewart, S., Toivonen, P.M.A., Moyls, A.L., 1999. Effect of warm, chlorinated water on the microbial flora of shredded iceberg lettuce. Food Res. Int. 32, 7–14.

Delaquis, P.J., Stewart, S., Cliff, M., Toivonen, P.M., Moyls, A.L., 2000. Sensory quality of ready-to-eat lettuce washed in warm, chlorinated water. J. Food Qual. 23, 553–563.

El-Kest, S.E., Marth, E.H., 1988a. Inactivation of *Listeria monocytogenes* by chlorine. J. Food Prot. 51, 520–524.

El-Kest, S.E., Marth, E.H., 1988b. Temperature, pH, and strain of pathogen as factors affecting inactivation of *Listeria monocytogenes* by chlorine. J. Food Prot. 51, 622–625.

Escudero, M.E., Velázquez, L., Di Genaro, M.S., De Guzmán, A.S., 1999. Effectiveness of various disinfectants in the elimination of *Yersinia enterocolitica* on fresh lettuce. J. Food Prot. 62, 665–669.

Flickinger, B., 1999. Quality drives a 'growing' industry. Food Qual. 6, 27–32.

Food Processors Institute, 1980. Cleaning and sanitizing. In: Katsuyama, A.M., Strachan, J.P. (Eds.), Principles of Food Processing Sanitation. The Food Processors Institute, Washington, DC (Chapter 4).

Francis, G.A., O'Beirne, D., 1997. Effects of gas atmosphere, antimicrobial dip and temperature on the fate of *Listeria innocua* and *Listeria monocytogenes* on minimally processed lettuce. Int. J. Food Sci. Technol. 32, 141–151.

Fukumoto, L.R., Toivonen, P.M.A., Delaquis, P.J., 2002. Effect of wash water temperature and chlorination on phenolic metabolism and browning of stored iceberg lettuce photosynthetic and vascular tissues. J. Agric. Food Chem. 50, 4503–4511.

Hurst, W.C., Schuler, G.A., 1992. Fresh produce processing—an industry perspective. J. Food Prot. 55, 824–827.

King, A.D., Magnuson, J.A., Török, T., Goodman, N., 1991. Microbial flora and storage quality of partially processed lettuce. J. Food Sci. 56, 459–461.

Li, Y., Brackett, R.E., Chen, J., Beuchat, L.R., 2001. Survival and growth of *Escherichia coli* O157:H7 inoculated ontCut lettuce before or after heating in chlorinated water, followed by storage at 5 or 15 °C. J. Food Prot. 64, 305–309.

Loaiza-Verlarde, J.G., Tomás-Barberán, F.A., Saltveit, M.E., 1997. Effect of intensity and duration of heat-shock treatments on wound-induced phenolic metabolism in iceberg lettuce. J. Am. Soc. Hort. Sci. 122, 873–877.

Mercer, W.A., Somers, I.I., 1957. Chlorine in food plant sanitation. Adv. Food Res. 7, 129–169.

Mermelstein, N.H., 1998. Minimal processing of produce. Food Technol. 52, 84–86.

O'Mahony, M., 1992. Understanding discrimination tests: a user-friendly treatment of response bias, rating and ranking *R*-index tests and their relationship to signal detection. J. Sens. Stud. 7, 1–47.

Peiser, G., López-Gálvez, G., Cantwell, M., Saltveit, M.E., 1998. Phenylalanine ammonia lyase inhibitors control browning of cut lettuce. Postharvest Biol. Technol. 14, 171–177.

Poste, L.M., Mackie, D.A., Butler, G., Larmond, E., 1991. Laboratory methods for sensory analysis of food. Agriculture Canada Publication 1864/E, 80 pp.

Simons, L.K., Sanguansri, P., 1997. Advances in the washing of minimally processed vegetables. Food Aust. 49, 75–80.

Toivonen, P.M.A., 1992. The reduction of browning in parsnips. J. Hort. Sci. 67, 547–551.

US Food and Drug Administration (USFDA), 1998. Guidance for Industry. Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables. US Food and Drug Administration, Washington, DC.

Wei, C.-I., Cook, D.L., Kirk, J.R., 1985. Use of chlorine compounds in the food industry. Food Technol. 39, 107–115.

Weissinger, W.R., Chantarapanont, W., Beuchat, L.R., 2000. Survival and growth of *Salmonella* baildon in shredded lettuce and diced tomatoes, and effectiveness of chlorinated water as a sanitizer. Int. J. Food Microbiol. 62, 123–131.