



Know-how for Horticulture™

Identification and quantification of hazards and risks to human health in the vegetable industry

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Media Summary

Until recently, food safety with respect to fresh produce was primarily concerned with pesticide residues. It was not commonly accepted that fresh produce could be associated with food borne diseases caused by microorganisms. However, outbreaks of food borne disease linked to the consumption of vegetables and fruit in developed countries is becoming more commonly reported. Consequently, the fresh produce industry has had to deal with a lot of new issues revolving around a new definition of food safety that includes human pathogens. With this have come a lot of challenges.

Uncertainty exists with regard to some of the technical aspects of on-farm food safety. There has been much confusion and many inconsistencies in the way systems have been implemented and audited because of a lack of information available on which to base these systems. This project originated to address these issues.

The major outcome has been the production of a national food safety guide that the vegetable industry can use as a reference tool. The guide covers all stages of vegetable production, highlights the risks involved for each input and makes recommendations to minimise those risks. Developed as a direct result of working hand in hand with an industry reference group, the guide will provide a practical point of reference on food safety. Subsequently, a similar guide was developed for the Victorian strawberry industry.

A farmgate survey of vegetables for human pathogens was carried out as part of this project. Whilst there are many studies published overseas looking at isolation of different human pathogens on whole fresh produce, this data cannot be found in Australia. We analysed around 200 vegetable samples from 35 farms in Victoria for a number of human pathogens. The vegetables chosen were salad types and include cos lettuce, salad mix, celery, cabbage and Dutch carrots. Overall incidence of pathogens found on the vegetable samples was low with one positive for *Salmonella victoria* and one for *Listeria monocytogenes*. Whilst it is preferable not to find such pathogens it is also encouraging that the numbers found were low, particularly when comparisons are made with overseas studies of this type.

Technical Summary

Until recently, food safety in fresh produce was primarily concerned with pesticide residues. It was not commonly accepted that fresh produce could be associated with food borne disease. However, outbreaks of food poisoning linked to the consumption of vegetables and fruit in developed countries is becoming more commonly reported. Consequently, the fresh produce industry has had to deal with a lot of new issues revolving around a new definition of food safety that includes human pathogens. With this have come a lot of challenges.

Uncertainty exists with regard to some of the technical aspects of on-farm food safety. There has been much confusion and many inconsistencies in the way systems have been implemented and audited because of a lack of information available on which to base these systems. This project originated to address these issues.

The major outcome has been the production of a national food safety guide that the vegetable industry can use as a reference tool. Subsequently, a similar guide was developed for the Victorian strawberry industry.

A desktop review and experimental work was undertaken to provide information on which to base the guide. This included challenge trials to study the effectiveness of chlorine as a postharvest wash, irrigation water analysis and soil analysis. A farmgate survey of vegetables for human pathogens was also carried out.

Whilst there are many studies published overseas looking at isolation of different human pathogens on whole fresh produce, this data cannot be found in Australia. We analysed around 200 vegetable samples from 35 farms in Victoria comprising of 5 different types of vegetables for *Salmonella* spp., *Listeria* spp., *E. coli*, *Campylobacter* spp. and faecal coliforms. Overall incidence of pathogens found on the vegetables sampled was low with one positive (0.5%) for *Salmonella victoria* and one (0.5%) for *Listeria monocytogenes*. Whilst it is preferable not to find such pathogens it is also encouraging that the numbers found were low and also that the levels detected were very low. A few samples (3.8%) were found to be positive for *E. coli*, mostly at low and acceptable levels.

The effectiveness of calcium hypochlorite on inactivation of *E. coli* inoculated on fresh produce was investigated. Different times of exposure and concentrations of chlorine were studied. Dipping was not effective at eliminating *E. coli* populations although it significantly reduced the *E. coli* counts compared to inoculated, undipped lettuce. Dipping inoculated cos lettuce leaves into hypochlorite solutions containing 50 mg/L or greater free chlorine for times of 30 seconds or greater reduced *E. coli* cells by approximately 1.9 to 2.8 log₁₀ colony forming units per gram (CFU/g) from an initial population of approximately 6.8 log₁₀ CFU/g. Dipping lettuce in water alone reduced cell numbers by 1.7 log₁₀ CFU/g. Dipping inoculated broccoli florets into hypochlorite solution reduced *E. coli* cells by approximately 1.7 to 2.5 log₁₀ CFU/g, depending on the time and concentration of the free chlorine in the wash water. Dipping broccoli in water alone reduced cell numbers by 1.5 to 1.8 log₁₀ CFU/g. Dipping broccoli florets for 2 minutes in a 100 mg/L free chlorine solution at temperatures between 4 and 25°C reduced *E. coli* cells by approximately 2.4 log₁₀ CFU/g. No significant effect of temperature on the level of cell reduction was observed.

Water samples were collected from seven farms in three main growing areas of Victoria, Werribee, the Mornington Peninsula and East Gippsland. Different water sources were looked at which included bore, dam, river and lake water. All of the samples except for one fell within the current Australian water quality guidelines of 1000 faecal coliforms per 100 mL. We found bore water to have much lower levels of faecal streptococci, faecal coliforms and *E. coli* than dam, river or channel water. This is not that surprising since surface water could come from some distance and there may be less control over potential sources of contamination. Most bore water samples had levels of faecal coliforms of less than 2 most probable number per 100mL (MPN/100mL), with the highest level being 14. Channel/river water samples contained from less than 2 to 350 MPN/100mL. Dam water had mainly between 5 to 540 MPN/100mL, with 2 samples containing 920 MPN/100mL faecal coliforms.

To enable limits to be set for *E. coli* in the selection of new land section of the 'Safe Vegetable Production' guide, soil samples were collected from a number of farms in Victoria and one in Queensland. Of the 188 samples taken *E. coli* was present in 23 (12.2%). In the remaining 165 samples *E. coli* was not detected. Of the samples where *E. coli* was found, 15 had levels of less than 50 CFU/g, and 2 had less than 100 CFU/g. Consideration of this data resulted in a recommendation that soil on new land should contain less than 100 *E. coli* (CFU)/g.

1. Introduction

Until recently, food safety in fresh produce was primarily concerned with pesticide residues. It was not commonly accepted that fresh produce could be associated with food borne disease outbreaks. However, outbreaks of food borne disease linked to the consumption of vegetables and fruit in developed countries is becoming more common. The number of documented fresh produce-related outbreaks in the USA more than doubled from between 1973 and 1987 to the period 1988 to 1991 (Tauxe *et al.*, 1997). Bacterial diseases have been attributed to *E. coli*, *Salmonella*, *Listeria*, *Shigella*, *Bacillus*, *Clostridium* and *Campylobacter* (Beuchat. 1995. Fain, 1996; Little *et al.*, 1997). Viruses and parasites have also been linked to produce-related disease outbreaks.

Consequently the fresh produce industry has had to deal with a lot of new issues revolving around a changing definition of food safety. Many quality assurance systems have been introduced which encompass food safety to address the risks involved in production. However, with this have come a lot of challenges. Uncertainty exists with regard to some of the technical aspects of on-farm food safety. There has been much confusion and inconsistencies in the way systems have been implemented and audited because of a lack of information available on which to base these systems.

This project originated to address these issues. A review was carried out early in the project looking at the microbiological hazards in the vegetable industry. This was submitted as a draft in an earlier milestone (number 2) but has since been finalised (Appendix 2).

The major outcomes of this project have been the production of food safety guides that the vegetable and strawberry industries can use as reference tools. The guides cover all stages of vegetable production, highlights the risks involved for each input and makes recommendations to minimise those risks.

2. Development of food safety guides

2.1 Safe Vegetable Production - A microbial food safety guide for the Australian vegetable industry

2.1.1 Consultation process for developing the guide

In developing the guide it was decided very early on that we should work very closely with the vegetable industry; firstly to ensure that it would be practical to use and therefore readily adopted and secondly that the industry would have some ownership of it. We enlisted a group of growers who were motivated and interested in being involved, many of whom are members of the Horticulture Australia industry advisory committees. We had regular meetings with these growers from early development of the guide through to its completion and their input was invaluable.

As the guide was developed it was sent out to a broader audience for comment. It was circulated to around 40 people from the broader horticultural community. This included researchers, extension officers, the supermarkets, the Australia New Zealand Food Authority (ANZFA), auditors in the industry, Agriculture, Fisheries

and Forestry Australia (AFFA), EPA Victoria, Australian Horticulture Corporation (AHC) and consultants. Whilst not all persons responded, there were representatives from each sector of the community who did respond and the comments received were very constructive in developing the guide further. The appropriate changes were made to the guide and it was sent out for further comment but at this time few changes were suggested.

Finally, it was sent to the Australian Vegetable and Potato Growers Federation and Industry development officers (IDO) in each state for them to get feedback from some of their growers.

2.1.2 Development of the guide

A review was initially carried out to identify the microbiological hazards that may be associated with fresh vegetables (Appendix 2). This included looking at overseas studies on the incidence of pathogens in fresh produce and outbreaks associated with fresh produce. Limited Australian data that could be found was included.

Desktop searches were carried out and contacts made for all areas of production to identify any existing information that could be applied or drawn on. Whilst this information was difficult to find, fragmented and diverse, there were some areas where such information was available and this was included.

Experimental work was carried out to supplement this information and this is detailed in this report.

Finally, the knowledge and experience of the food safety group was drawn on for areas where information was not really available to make some of the recommendations.

2.1.3 Format and publishing of the guide

The guide was designed and published in a binder style to allow for the addition of new or revised information, as it becomes available. Its format is easy to use and read with sections covering each stage of vegetable production, starting with land selection through to transport after leaving the farm. It also includes a section on produce testing which has been one of the areas of great debate.

For each section the guide discusses the hazards involved with the particular input, for example irrigation water quality or hygiene of workers, and then states any facts that can be drawn on. This is followed by the industry recommendations to minimise the hazards. The guide is unique in that it is very specific and prescriptive and therefore provides answers that the growers, as well as auditors, have been asking for.

The guide was officially launched by Dr Jane Wilson at Hort 17, a horticultural field **event** held in Gatton, Queensland, in May this year. It has been submitted to HAL as

A separate publication (milestone number 5).

2.2 Safe Production of Strawberries - A guide to minimise microbial food safety hazards for the Victorian strawberry industry

Work on the Safe Production of Strawberries guide began in June 2001. This was added on to the project when additional funding was provided by the Victorian Strawberry Industry. The guide identifies the microbial food safety hazards that may exist during the primary production of strawberries. It does not include distribution at the retail level or handling and preparation in the food service sector or in the home. It is modelled on the Safe Vegetable Production guide since many areas such as irrigation water quality, hygienic handling, etc have common issues. As with the vegetable guide it is designed for operations to use in conjunction with a HACCP based Quality Assurance System.

The purpose of the guide is to describe:

- the sources of microorganisms that can cause foodborne disease in strawberries.
- the conditions that favour the growth and survival of these organisms in strawberries.
- recommendations that may prevent or minimise contamination during the growing, harvesting, packing and transporting of strawberries.

2.2.1 Consultation process to develop the guide

Members of the Victorian strawberry industry and the Strawberry Industry Development Officer were included in the development of the guide. A range of strawberry growers from small to large enterprises were interviewed and asked questions in regard to each stage of production. They were asked about the growing environment, the inputs, the equipment and facilities, the staff and potential hazards that they felt might exist.

The guide did not need to undergo the same extensive consultation process as for the vegetable guide, since many issues were resolved by that process and this didn't need to be repeated. The guide was edited by staff at NRE including those involved in strawberry research and members of the Victorian strawberry industry. A draft was presented to growers at a Victorian Strawberry Growers Association meeting in April 2002 for comment and it was extremely well received.

The guide has been published in a brochure style that the Victorian strawberry industry wants to add to The Victorian Strawberry Industry Resource Manual. It has been designed in a user-friendly format, with illustrative photographs throughout. It is submitted as a separate publication to this report.

The guide will be officially launched in August 2002 at the Victorian Strawberry Industry's annual general meeting.

2.1.2 Poster preparation

The second component of this work was to design a poster on food safety to reinforce training messages for workers in the Victorian strawberry industry. The poster can be displayed inside the strawberry packing shed and is pertinent to all workers on the strawberry farm. The strawberry HX) was involved in the discussions on the content of the poster. The poster is composed of pictures (in this

case photographs) and simple statements reminding workers of important food safety considerations. The statements are printed in English as well as in Italian, Vietnamese and Cambodian, the native languages of many of the workers on the farms. The hazards described in the poster are as follows:

- always use the toilets provided
- always wash hands before starting work and after the toilet, eating or blowing your nose
- always handle fruit carefully
- cover sores, cuts with bright bandages and gloves if on hands
- if ill let your supervisor know
- keep work surfaces clean

The poster was then given to graphic designers to complete the artwork and the file has been given to the strawberry IDO for their use. It is submitted on disk as a separate appendix to this report.

3. Vegetable farmgate survey

3.1 Introduction

Whilst there are many studies published overseas looking at isolation of different human pathogens on whole fresh produce, this data is not readily available in Australia. Therefore, it was decided to undertake a study on Australian produce, as this is an unknown area here. Such a study provides an indication of how well farm practices are working to minimise food safety hazards. In addition, collection of samples from the farmgate provides information on practices on-farm as opposed to what might happen once the produce reaches distribution and marketing.

3.2 Methodology

3.2.2 Vegetables surveyed

Vegetables chosen for the survey were cos lettuce, cabbage, celery, salad mix and Dutch (baby) carrots. These were chosen on the basis **that** they are normally eaten without being cooked or can be eaten raw. In addition these crops are grown close to or in the ground and some (eg. Salad mix, lettuce, cabbage) have uneven, large surface areas where microorganisms may attach and be protected. All of these factors place these vegetables into a higher food safety risk category than those that are typically cooked before consumption or are grown off the ground.

3.2.3 Farms

Farms selected for the survey were in Wembree South, Bacchus Marsh, Keilor, Oaklands Junction and the Mornington Peninsula (Boneo, Clyde, Pearcedale, Cranbourne, Somerville, Dandenong, Tyabb, Rosebud, Devon Meadows, Fiveways, Heatherton, and Keysborough). In total 35 farms were involved, with 8-10 farms chosen for each vegetable type. Farms provided 1, 2 or 3 different types of the selected vegetables.

Each grower was questioned on practices used on the farm. The questionnaire is shown in Appendix 1.

3.2.4 Sampling

Each farm was visited twice to collect samples over summer and autumn and in some cases a third visit was carried out in spring. At each collection two samples were analysed from each farm for each produce type.

Two boxes of celery or cos lettuce were collected from each farm, with each box being one sample. For celery, four stalks were removed from five bunches. The lowest 2cm of the stem and the leaves were removed and discarded and the remainder was chopped and mixed. 100g was taken as the sample. In the case of lettuce, the outer, damaged leaves were discarded and then four leaves were taken from five lettuce heads. 100g was taken as the sample.

One box of salad mix was collected and two 50g samples taken from the mix.

Ten bunches of Dutch carrots were collected, with five bunches making up one sample. Five or six carrots, depending on size, were removed from each bunch, chopped and mixed, and 100g was taken for the sample.

Eight cabbages were collected, with 4 making up one sample. A few of the older, outer leaves were removed and discarded and then the cabbages were quartered. A section from each quarter was cut off, with different parts of the cabbage selected from each quarter. Sections were selected in this way for each of the four cabbages and then chopped and mixed. 100g was taken for the sample.

Produce was stored overnight at 2-4°C prior to being analysed.

3.2.5 Microbiological analysis

~~Each sample was analysed for *Listeria* spp., *Salmonella* spp., *E. coli*, faecal coliforms and total aerobic counts for the summer and autumn collections. In addition the summer collection samples were analysed for *Campylobacter* spp.~~

~~The samples collected in spring were analysed for *Listeria* spp., *Salmonella* spp., *E. coli* and *Campylobacter* spp.~~

3.2.5.1 *Listeria*

~~Samples were stomached for 2 minutes in 225 ml half Fraser broth (Oxoid) and plated onto Oxford agar (Oxoid). The plates were incubated at 37°C for 48 hours. Following plating, the bags were incubated at 30°C for 24 hours. A second enrichment was carried out by transferring 0.1 ml to 10 ml Fraser broth and incubating at 37°C for 48 hours. These samples were plated onto Oxford agar and if typical colonies were found the plates were sent to the Microbiological Diagnostic Unit, Melbourne for confirmation and identification. Positive and negative control organisms were taken through the same procedure. The organisms used were *L. innocua* 2305 and *Staphylococcus aureus* ATCC 25923.~~

3.2.5.2 *Salmonella*

~~Samples were stomached for 2 minutes with 250 ml Wc bacto peptone. They were then plated onto XLD agar and incubated for 24 hours at 37°C. After samples were plated the bags were incubated for 16-20 hours at 37°C, the samples were then transferred to mannitol selenite cystine broth (Oxoid) and incubated at 37°C for 24 hours. The enrichment broth was plated onto XLD agar and incubated as above. If typical colonies were found the plates were sent to the Microbiological Diagnostic Unit, Melbourne for confirmation and identification. Positive and negative control organisms were taken through the same procedure. The organisms used were *S. salford* IMB 1740 and *Citrobacter freundii* NCTC 9750.~~

3.2.5.3 *E. coli* and faecal coliforms

~~Samples were stomached for 2 minutes with 250 ml tryptone soya broth. They were then »ere plated onto 3M Petrifilm *E. coli*/coliform count plates and incubated in a water bath at 44-44.5°C. The bags were incubated for 24 hours at 37°C for enrichment and plated and incubated in the same way.~~

4. Effectiveness of chlorine to remove pathogens from vegetables

4.1. Introduction

Hypochlorite dips are commonly used for washing fruits and vegetables after harvest, particularly in the fresh-cut industry. Washing reduces the total microbial load and in so doing may reduce spoilage and maintain quality, thereby increasing the shelf life. Another important reason to wash produce in sanitised water is to increase product safety. There is often a general assumption that washing in sanitised water will eliminate pathogenic organisms that may be present. However, little published data is available to support this.

Of the few studies that have been carried out, most have investigated the effect of chlorine on the inactivation of *Listeria monocytogenes* (Brackett, 1987; El-Kest and Marth, 1988; Beuchat and Brackett, 1990; Zhang and Farber, 1996). *In-vitro* testing of chlorine against *L. monocytogenes* has shown it to be an effective sanitiser (Zhang and Farber, 1996). Its effectiveness on vegetables has not been shown to be particularly good. Chlorine washing was found to reduce *Listeria monocytogenes* populations on lettuce and Brussels sprouts by only 2 logio CFU/g or less (Brackett, 1987; Beuchat and Brackett, 1990; Zhang and Farber, 1996). Furthermore, the initial reduction observed on lettuce was not evident when compared to controls after 15 days storage at 5°C (Beuchat and Brackett, 1990). Zhuang *et al.* (1995) looked at the effect of chlorine on *Salmonella montevideo* inoculated on tomatoes. Chlorine was found to reduce populations by around 1 logio CFU/g.

The presence of the coliform *E. coli* is often used as an indicator of faecal contamination and the possible presence of pathogens. There have been several human disease outbreaks overseas associated with fresh produce caused by enterotoxigenic and enterohaemorrhagic *E. coli* and other faecal organisms such as *Salmonella*, and *Campylobacter* (Beuchat, 1995; Little *et al.*, 1997). There is increasing pressure being placed on primary producers to ensure that produce is safe for human consumption. The introduction of HACCP based quality assurance programs and the emphasis on food safety has meant that chlorination is used more and more as a tool to satisfy HACCP requirements. The main aim of this study was to look at the effect of various chlorine concentrations and contact times on the fate of *E. coli* inoculated on fresh produce.

4.2 Materials and methods

4.2.1 Preparation of the *E. coli* suspension

A modified *E. coli* (strain TGI) was used for this study. The *E. coli* were cultured each week on luria bertani broth (LB) agar (containing 1µl/ml ampicillin). For each experiment, one loopful of the culture was inoculated into a flask with LB (containing 1 µl/ml ampicillin) and incubated with shaking for 24h at 35°C. The concentration of this stock suspension was confirmed by making serial dilutions in peptone buffer containing 0.1% bacto peptone (Difco, Detroit, USA) in deionised water. These dilutions were plated onto Petrifilm *E. coli*/coform count plates (3M

5. Irrigation water and soil analyses

5.1 Introduction

Current Australian water quality guidelines (Australian and New Zealand Environment and Conservation Council, 1992) recommend that irrigation water should contain not more than 1000 faecal coli forms/100mL. There was some suggestion that replacement guidelines might recommend less than 10 faecal coliforms/100mL for some crops. As there was no data available to suggest whether this was a reasonable recommendation or whether irrigation waters adhere with current guidelines, we carried out some sampling in different growing regions of Victoria.

To enable limits to be set for *E. coli* in 'the selection of new land' section of the 'Safe Vegetable Production' Guide, soil samples were collected from a number of farms in Victoria and one in Queensland.

5.2 Irrigation water

5.2.1 Methodology

5.2.1.1 Water sources

Water samples were collected from seven farms in three main growing areas of Victoria; Werribee, the Momington Peninsula and East Gippsland. Farms in East Gippsland were in Lindenow, Stratford and Boisdale and on the Momington Peninsula, in Somerville and Clyde.

Different water sources were looked at, these included bore water, dam, river and lake water.

Farm 1 had dam water made up of rain and bore water.

Farm 2 had dam water that consisted mainly of catchment from farm run-off with some bore water added.

Farm 3 had dam water distributed via channels from the Werribee river.

Farm 4 had dam water distributed via channels from the Werribee river, except for the autumn measurement when water was supplemented from the D1 drain run-off. This is catchment water from the surrounding area. This farm also had bore water.

Farm 5 accessed water directly from the channel that originated from Lake Glenmaggie.

Farm 6 pumped water direct from the river Avon and also had bore water

Farm 7 pumped water direct from the Mitchell river and also had bore water.

~~5.2.1.2 Sampling and analysis~~

~~Sampling was carried out during each season.~~

~~Around 200mL of water was collected into a sterile bottle for each sample. This was transported under ice back to the laboratory and stored overnight at 4°C prior to analysis. Analysis was carried out within 24 hours as specified in the Australian standard AS 1095.4.1.1.~~

Table 23. Soil measurements taken in Queensland during April 2000

Farm 1			Farm 2		
Sample	<i>E. coli</i>	Total coliforms	Sample	<i>E. coli</i>	Total coliforms
CFU/g			CFU/g		
A1	ND	56	A1	ND	6
A2	ND	1.1×10^2	A2	ND	11
A3	ND	1.1×10^2	A3	ND	11
A4	ND	11	A4	ND	ND
A5	ND	39	A5	ND	ND
A6	ND	5.8×10^2	A6	ND	ND
A7	ND	1.5×10^2	A7	ND	ND
A8	ND	7.7×10^2	A8	ND	ND
B1	ND	36			
B2	ND	1.3×10^3			
B3	ND	9.0×10^2			
B4	ND	61			
B5	ND	38			
B6	ND	6			
B7	ND	74			
B8	ND	3.3×10^3			

ND = not detected

6. Technology transfer

The guide was published and launched as described (section 2.1.3). The launch and availability of the food safety guide has been publicised widely. Flyers with details about the guide were given to attendees at the National lettuce conference held in May 2002. Press releases have been sent to the EDOs to advertise in their newsletters and articles have been published in the newsletters IHD links (no. 6, May 2002) and Vegetable matters (no. 4, April 2002) and the journals Fruit and Vegetable news (June 2002) and Good Fruit and Vegetables (July 2002).

The release was also sent to around 30 different newspapers. Some of these have used the article and it has generated enquires about the guide. Flyers were sent to Tasmanian Quality Assured to include in their satchel for an On-farm Food Safety conference held in July 2002.

The launch and articles published to date have led to many enquires from growers, auditors, education establishments and exporters requesting a copy of the guide.

Another project led by Swinburne Tafe resulted in a video for poor language skill workers to be produced based on the content of the guide. This is for growers to use to train their employees. The video and training manual package was launched in November 2001.

There have been a number of presentations at conferences and in printed format during this project. Results of work on the effectiveness of chlorine washing in particular has been published in industry journals as well as in the scientific community. A one-page leaflet on this work was distributed widely to individual growers via the IDOs in each state. Around 1000 copies were sent out.

6.1 Publications related to the project

Behrsing, J., Winkler, S., Franz, P. and Premier, R. (1999) Inactivation of *Escherichia coli* on vegetables by chlorine. Australasian Postharvest Horticulture Conference, 3-8 October, Waitangi, New Zealand, (poster)

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