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New washing treatments for minimally processed vegetables

Lloyd Simons, et al
Food Science Australia

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New Washing Treatments for Minimally Processed Vegetables

Mr Lloyd Simons, et al.

Food Science Australia

VG 97045 (October, 2000)

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Project Objectives:
To provide the fresh-cut vegetable industry with a chemical and/or physical alternative to chlorine, for the washing of fresh-cut produce.

Funding Sources:
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COOPERATIVE RESEARCH CENTRE FOR INTERNATIONAL FOOD MANUFACTURE & PACKAGING SCIENCE

FOOD SCIENCE AUSTRALIA
A JOINT VENTURE OF CSIRO AND AFISC

Media Summary

This project has been able to provide a viable chlorine-alternative for washing fresh vegetables. The new method and overcomes the major limitations fresh-cut vegetable processors were encountering with using chlorine. The commercial trials with the new sanitiser identified several advantages for processors and consumers. The alternative sanitiser eliminates potential chlorinated by-products, reduces gas-off for workers in the washing environment. The new method also has improved stability under high organic load conditions, has no requirement for pH adjustment and the new method can be promoted as a very suitable alternative to chlorine. The new method gives comparable performance to chlorine for reducing bacterial numbers on fresh-cut produce, including any present harmful bacteria such as *Listeria monocytogenes*.

The industry adoption of the new method to date has illustrated the success of this achievement and the broader implications for the horticultural industry. Two years ago, all processors used chlorinated washing water. As a result of this project one major operator is using the non-chlorine technology at both of it sites. A further three medium sized processors are also using the non-chlorine technology. We estimate that approximately 40% of fresh-cuts industry (by turnover) has changed its washing procedures as a result of this project. Food Science Australia has made presentations to and is in discussion with about a further 30% of the industry. Indications are that they are seriously considering changing their washing technology too. We believe that the remainder in the industry will follow in 12-24 months.

Technical Summary

Pilot scale fresh-cut vegetable washing trials were conducted at the Food Processing Centre (Food science Australia, Werribee) to evaluate 4 potential chlorine alternatives for the purpose of replacing chlorine as the sanitising agent in the produce washing system. These included, (i) iodine releasing resin, (ii) ozone, chlorine dioxide and hypochlorous acid (mixed oxidant system), (iii) peracetic acid and hydrogen peroxide and (iv) silver ions and hydrogen peroxide system.

Overall, a combined peracetic acid and hydrogen peroxide treatment was the most effective chlorine alternative for the reduction in total microbial populations and reduction of pathogens of concern to food safety. The sanitiser system can be conveniently dosed and monitored in a similar way to chlorine, and is suitable for spraying, fast flumes and washing tanks applications.

The MCV resin system was also found to be potentially suitable alternative as it is none corrosive and convenient to use. The system available for this trial had limitations relating to the concentration of released iodine, which limited the microbiological reduction achieved. Use of higher water temperature water was required too increase the release of iodine to provide acceptable microbiological reduction, this necessitated heating and re-cooling of the water. In addition, iodine will require registration in the ANZFA Food standards and/or National Registration Authority before this application can be allowed.

The mixed oxidant system and the silver / hydrogen peroxide system did not satisfy functional requirements, particularly if used in a recycling washing system. In addition considerable capital cost is required for these systems, which will limit implementation by small operations. The mixed oxidant system provided only a partial reduction in chlorinated by-products, which is not as attractive as a chlorine free system. The silver ion and hydrogen peroxide system did not provide satisfactory microbiological reduction and the accumulation of silver residue on the produce was an impediment. Silver ions will also require registration in the ANZFA Food standards and/or National Registration Authority before this application can be allowed.

Based on our research findings, the peracetic acid and hydrogen peroxide sanitiser was recommended for industry adoption as a chlorine alternative for washing fresh-cut vegetables. The commercial trials with a peroxyacetic acid and hydrogen peroxide sanitiser identified several advantages (identified in pilot-scale trials) for processors and the public. The alternative sanitiser eliminated chlorinated by-products which are of public health and environmental concern, reduced gas-off which is an OH&S concern for some fresh-cut processors when washing with chlorine in confined areas. Other advantages included improved stability under high organic load conditions, no requirement for pH adjustment and provided potential for promoting chlorine free processing.

The ultrasonic washing was demonstrated to provide a significant decrease (<1 log) in the total viable count of bacteria on shredded lettuce in non-sanitised wash water and in wash water dosed with chlorine or peracetic acid/hydrogen peroxide. The performance of ultrasonics is critically dependent on the capacity to maintain cavitation in the washing water, this was impeded by absorption of the cavitational energy by the produce (particularly when overloading water washing tank) and by using chilled washing water (ie. 4°C). Under such conditions ultrasonics was not effective. Furthermore, prolonged treatment produced physiological damage at higher treatment temperatures, which resulted in earlier softening of produce during shelf-life storage trials.

Introduction

Key drivers for consumers in their choice of foods include:

- Convenience, foods that are fast to prepare;
- Healthy, foods that are fresh and unprocessed, and
- Safe, foods being free from microbiological, physical and chemical contamination

Fresh-cut salads meet these choice drivers and are reflected in increased sales in both the retail and the food-service industry. These products comprise a range of differing whole lettuce varieties, shredded lettuce, broccoli florets, carrot battens and a range of different vegetables suited for preparation of green salad, stir-fry or soup products.

These short shelf life, chilled, fresh products do not include a processing step that will eliminate pathogenic micro-organisms that may be present and can cause ill health in humans. Overseas experience has been that product washing is an important step in removal of physical contaminants such as soil, and for reducing the microbiological loading that is found naturally on these fresh foods. Microbiological populations are reduced when chlorine is used as a sanitiser in the water.

Due to the importance of product washing, the Cooperative Research Centre for International Food Manufacture and Packaging Science, of which NRE and CSIRO are both foundation members, took an interest in this field. They established a fresh-cut forum in 1997, to develop guidelines on behalf of the fresh-cut industry for the production, washing and distribution of their products. The forum included 6 industry participants, which represented in excess of 90% of the Australian Fresh-cut industry, plus the 3 main supermarket retailers.

This forum recognised the need of an alternative to chlorine for the sanitised washing of fresh-cuts. Fresh-cut processors had identified several limitations with the use of chlorine: (1) sanitation efficacy: it is rapidly depleted under high organic loading encountered in vegetable washing which creates problems for process control systems to maintain a effective level in the water; (2) requirement for pH adjustment of the wash water to maintain optimum performance; (3); OH& S problems due to gassing – off of chlorine during processing; and (4) environmental and public health issues relating to the potential production of chlorine by-products in wash-water. It also was evident that the international trend was for the eventual phasing-out of chlorine, provided suitable replacements could be implemented and consequently several alternatives were beginning to be developed, evaluated and commercialised overseas.

The CRC-IFM&PS responded to the fresh-cut industry needs by establishing this project with the purpose to identify, evaluate and implement effective washing systems that do not use chlorine as the active sanitiser and that meet the Australian and New Zealand Food Standard Codes.

Materials & Methods

A project plan was developed that would learn from international experiences in research and industry experience in the fresh-cut industry, and other allied fields of product sanitation.

1. Review of international 'best practice' washing technologies in the fresh-cut industry
2. Identifying chlorine alternatives, used in either the fresh-cut industry or allied industries, assessing their likelihood of improving on the current systems, and prioritising the research program
3. Conducting preliminary laboratory screening and assessment of high priority alternatives
4. Scale-up, development and validation trials at pilot-scale trials at Food Science Australia's Food Processing Centre (Werribee)
5. Preparing a cost/benefit assessment
6. Industry consultation and demonstration phase
7. Conduct of demonstration trials with Industry
8. Facilitating industry adoption

An international survey of sanitisers and washing systems was undertaken to identify potential chlorine alternatives. Preliminary screening of washing systems based on microbiological efficacy, which was undertaken to efficiently narrow the focus of activities to the best possible alternatives.

Pilot-scale washing trials were conducted at the Werribee processing facility on 4 chlorine alternative systems, which included (1) mixed oxidant system (a combination of ozone , chlorine dioxide and hypochlorous acid), (2) peracetic acid and hydrogen peroxide, (3) iodine, (4) silver ions and hydrogen peroxide, and (5) ultrasonic treatment. The systems were evaluated for microbiological efficacy under various conditions that effect performance, such as contact time, temperature, pH and organic load. Comparison of the systems also included consideration of effective and convenient monitoring and dosing control for implementation in HACCP-based food safety programs. Preliminary evaluation of the sanitisers against pathogens of public health concern (ie *Listeria monocytogenes, Salmonella spp. and E.coli*) was also also included in the selection process.

Consultation was conducted with the main participants in the Fresh-cut industry (in Vic, NSW, Qld), to convey research findings from the trials at Werribee and to establish commercial trials. Industry consultation was conducted through the fresh-cut forum and directly with the three major industry participants which included Salad Fresh (Vic), Keilor Valley Gardens (Vic), Harvest Fresh-cuts / Vegco (Vic), Mrs Crockets Kitchens (Qld), Harvest Fresh-cuts (Qld), All States (NSW), and Moriatis (NSW).

Commercial trials were conducted in Victoria at Keilor Valley Gardens and facilitated in Queensland at Harvest Fresh-cuts. This was conducted to validate findings under commercial conditions and to facilitate technology transfer.

Pilot-scale ultrasonic washing trials were conducted at the Food processing Centre (Food Science Australia, Werribee), as a physical alternative to chemical sanitation or to enhance the performance of sanitised washing. Ultrasonic washing was performed on shredded lettuce, sliced carrot and broccoli florets in a 20 litre tank, with different wash water temperatures, treatment periods, ultrasonic power out-puts and sanitiser concentrations for peracetic acid/hydrogen peroxide and chlorine.

The methodology for all the pilot-scale and commercial trials summarised above, are contained in technical reports submitted to the CRC, NRE and HRDC, which are listed in the appendix.

Results

Overall, peracetic acid and hydrogen peroxide was the most effective chlorine alternative based on the microbiological criteria, for reduction in total microbial populations and reduction of pathogens of concern to food safety. This sanitisers can be conveniently dosed and monitored in a similar way to chlorine, and was applicable for spraying, fast flumes and washing tanks.

The iodine system was also a favourable alternative as it is non corrosive and convenient to use. The system available for this trial had limitations relating to the concentration of released iodine, which limited the microbiological reduction achieved. Use of higher water temperatures required to increase the release of iodine to provide acceptable microbiological reduction could be achieved, however this necessitats heating and re-cooling of the water. Iodine will require registration in the ANZFA Food standards and/or National Registration Authority before this application can be allowed.

The mixed oxidant system and the silver / hydrogen peroxide system did not satisfy functional requirements, particularly if used in a recycling washing system. In addition considerable capital cost is required for these systems, which will limit implementation to small operations. The mixed oxidant system provides only a partial reduction in chlorinated by-products, which is not as attractive as a chlorine free system. The silver ion and hydrogen peroxide system did not provide satisfactory microbiological reduction and the accumulation of silver residue on the produce was an impediment. The use of silver ions will require registration in the ANZFA Food standards and/or National Registration Authority before this application can be allowed.

The peracetic acid and hydrogen peroxide sanitiser was recommended for industry adoption as a chlorine alternative for washing fresh-cut vegetables. Commercial trials were conducted in Victoria at Keilor Valley Gardens and facilitated in Queensland at Harvest Fresh-cuts. The commercial trials verified several advantages (identified in pilot-scale trials) for processors and the public in adoption a chlorine alternative for washing fresh-cuts The alternative sanitiser eliminated chlorinated by-products which are of public health and environmental concern, reduced gas-off which was an OH&S concern for some fresh-cut processors when using washing with chlorine in confined areas, had improved stability under high organic load conditions, and required no pH adjustment. It also provided potential for promoting to consumers chlorine free processing, if that was thought to be desirable.

The ultrasonic washing provided a measurable decrease in the total viable count on shredded lettuce in non-sanitised wash water and in wash water dosed with chlorine or peracetic acid/hydrogen peroxide. However, this increase was generally of marginal practical value at less then 1 log. The performance of ultrasonics is critically dependent on the capacity to maintain cavitation in the washing water, this was impeded by absorption of the cavitational energy by the produce (particularly when overloading water washing tank) and by using chilled washing water (ie. 4°C). Under such conditions ultrasonics was not effective. Furthermore, prolonged treatment produced physiological damage at higher treatment temperatures, which resulted in earlier softening of produce during shelf-life storage trials.

The technical details of the results for all the pilot-scale and commercial trials summarised above, are contained in technical reports submitted to the CRC, NRE and HRDC, which are listed in the appendix.

Discussion

The peracetic acid / hydrogen peroxide sanitiser has been proven to be a viable alternative to chlorine for washing in fresh-cut operations. Strong interest was and is received from fresh-cut processors, which was and is driven by a variety of processing, public health, environmental and marketing reasons. The priority of which differs depending on the size, type and market focus of each operation. Providing, comparable microbiological reduction was achieved with the chlorine alternative, the other major processing factors influencing the decisions for implementation were, improved process control with greater stability under high organic loading and occupational health and safety benefits due to reduced gassing–off from the wash water.

The application of sanitisers in vegetable washing systems always requires validation to optimise washing performance, due to the differences in washing system design (ie sprays, flumes, tanks), process operation (ie. batch, recycling, flow-through), variety of produce and water quality. Validation will need to include microbiological testing on product before and after washing to determine reduction in total viable count and other targeted populations, and testing of the water for microbiological quality, available sanitiser, water temperature, pH and organic loading. This will provide appropriate information to establish washing conditions most suitable to meet processing objectives. These practices form essential critical control points for HACCP based food safety programs. Optimisation of sanitised washing practices is also critical for shelf-life extension. Effectively reducing the microbiological population to low levels prior to packaging, will extend the growth period required before spoilage levels are achieved. Appropriate process control also offers potential for quantifiable risk assessment by the implementation of Food Safety Objectives. This is based on the knowledge of pathogen incidence and levels in raw product, reduction during sanitised washing, potential for growth during processing and storage prior to consumption. Survey of fresh-cut produce (supported by CRC, DNRE(Vic) and HRDC) and industry discussion has identified the major pathogens of concern for the fresh-cut industry *are Listeria monocytogenes, E.coli and Salmonella spp.* Further research is required to validate and optimise the reduction of these pathogens on fresh-cuts, which is required for development of quantifiable Food Safety Objectives.

Laboratory scale and pilot-scale trials demonstrated the potential for ultrasonic washing to increase the reduction in total viable count on fresh-cut produce. Commercial development of ultrasonic washing for fresh-cuts will be limited by absorption of the cavitational energy by the produce and the reduced effect in chilled washing water. Development of ultrasonic washing equipment to maintain adequate cavitational energy will be critical to providing a system that could be suitable for fresh-cut washing applications. This will require collaboration with a major manufacturer of ultrasonic equipment to develop a prototype for further trials. They need to provide expertise during scale-up for optimising ultrasonic cavitational energy.

Technology Transfer

The Australian fresh-cut industry has been estimated at $100m plus for 2000-01 (industry based estimate), this figure includes food service and retail for the domestic market. The growth of the industry for the last 2 years has been in excess of 10% and is expected to maintain that rate of growth at least for the short-term (industry based estimate). In this fast growing and dynamic industry, technology change is fast. Change occurs rapidly in both the two major domestic producers, plus about 15 smaller producers. Two years ago, all processors used chlorine based washing. As a result of this project one major operator is using the non-chlorine technology at its Brisbane site, and is in the process of switching to non-chlorine technology at its substantial major plant at Bairnsdale. At least three further medium sized processors are using the peracetic acid & hydrogen peroxide technology in Victoria. We estimate approximately 40% of fresh-cuts industry (by turnover) has changed its washing procedures as a result of this project. Food Science Australia has made presentations and is in discussion with about a further 30% of the industry, and indications are that they are seriously considering changing their washing technology too. We believe that others in the industry will follow in 12-24 months.

The chlorine alternative has been adopted by the operators involved in the commercial trials, and is currently being trialed and implemented by other processors. Direct industry transfer is regularly provided to enquires from processors and growers who wash their produce, needing information on what alternative sanitisers can be used and recommended dosing and monitoring procedures.

Further benefits have been identified for other horticultural produce (ie. fruits and vegetables), through discussions with growers, processors, and HRDC program managers. Project development is being undertaken to broaden the benefit of the technology outcomes to the wider horticultural industry.

Recommendations

- Validation and optimisation of pathogen reduction (Listeria monocytogenes, E.coli and Salmonella spp.) for the development of Food Safety Objectives, to provide quantifiable risk management options for fresh-cut operations.

- The industry adoption has illustrated the success of this achievement and the broader implications for the horticultural industry. The findings and benefits demonstrated during this project should be extended to other horticultural commodities. Further industry development should also be supported financially by the commercial suppliers of the sanitisers.

Acknowledgments

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Bibliography

Simons.L.K. and Sanguansri.P. (1997) Advances in the washing of minimally processed vegetables. Food Australia 49 (2) 75-80.

Simons.L.K. (2000) Evaluation of Tsunami as an alternative to chlorine for the washing of shredded lettuce. Poster, 33rd Annual AIFST Conference.

Appendix

Milestone reports submitted to CRC, DNRE(Vic) and HRDC on the washing of minimally processed vegetables

1. Review of washing technologies for minimally processed vegetables, December 1996
2. Pilot-scale washing trial of minimally processed vegetables using MCV-resin technology, December 1997
3. Pilot-scale washing trial of minimally processed vegetables using Tsunami™, April 1998
4. Pilot-scale washing trial of minimally processed vegetables using a mixed oxidant system, June 1998
5. Pilot-scale washing trial of minimally processed vegetables using a silver / hydrogen peroxide system, June 1998
6. Cost-Benefit Analysis for chlorine alternatives to sanitise fresh-cut vegetables, June 1998
7. Industry consultation, December 1998
8. On-line monitoring and dosing control for Tsunami vegetable washing system, December 1998
9. Evaluation of chlorine and Tsunami washing systems for lettuce salads at a commercial processing facility, February 1999
10. Preliminary investigation of ultrasonic washing for minimally processed vegetables, March 1999
11. Evaluation of ultrasonic washing for minimally processed vegetables, June 2000

Milestone #1: Review of washing technologies for minimally processed vegetables.

1. INTRODUCTION

1.1 Objectives

The washing process is an integral step in the production of minimally processed vegetables (MPVs) (see figure 1). Washing generally involves the immersion of product into a water bath, of cooled-chlorinated water. This is usually followed by a de-watering step to remove surface water and vegetable juice from the product, thus minimising microbial growth. The objective of washing is to remove debris, reduce the microbial load, and lower the product temperature.

The washing water becomes increasingly spoiled with vegetable juices and particles after continuous washing. Consequently the washing effectiveness is reduced, unless large volumes of water is discarded and replaced. For a continuous clean-water operation, which minimises water wastage and maintains washing effectiveness, the water should be cleaned and recycled. Continuous monitoring of the washing system (eg. sanitiser, pH) is also important to ensure washing effectiveness (see figure 2).

The objective of this document is to review existing technology and explore new advances which may have the potential to improve the washing of minimally processed horticultural products. The target products being lettuce, broccoli, and carrots. This will involve a literature search and an industry survey of equipment and chemical suppliers.

Types of washing, dewatering and recycling systems will be discussed with respect to design types, features, and product suitability. The use of alternative or additional washing agents shall be discussed with respect to their washing performance in reducing microbial populations and physiological disorders. This will include the integration of these chemicals into a system and the monitoring of factors required to ensure their effectiveness.

This report will supply recommendations for the improvement of washing / dewatering / recycling / monitoring systems, and washing chemicals that can enhance washing effectiveness, achieving an advantageously extended shelf-life of size reduced fresh horticultural produce.

1.2 Elements of washing systems

The efficiency of washing can be improved by the pre-cleaning of product prior to washing. This, typically includes, coring and removal of outer leaves of lettuces, the removal of carrot ends and brushing, and general trimming and discarding of any damaged or spoiled product (see figure 1).

A washing system requires the immersion of product in water for the removal of dirt, pests, sand, and micro-organisms. Human pathogens found in minimally processed vegetables and spoilage micro-organism are summarised in table 1.

Designs may include closed flumes (eg. pipes), open flumes (eg. channels), baths and wash-tanks. Agitation of the wash water is important to ensure adequate contact to surfaces and removal of debris. Agitation or mixing of the wash water can be achieved by a pump, water jets, air jets and gravity flow. Each type of wash system offers different solutions with respect to product suitability, washing intensity (turbulence), capacity and cost. These considerations should be addressed in the selection of a suitable wash system for a production line.

The washing system provides an opportunity to initiate or maintain the cold chain by lowering product temperature with the use of water-cooling systems and iced water. This is important with respect to the control of microbial growth and enzymatic activity which may result in physiological disorders (eg. browning) and increased product respiration.

Many washing systems include a sanitiser which is usually sodium hypochlorite (see 2.1). More recent developments include the application of chlorine potentiator to improve sanitation efficiency, and other alternatives like ozone and chlorine dioxide. Dipping solutions containing antimicrobials, antioxidants and antibrowning agents have also been developed to control physiological changes and microbial growth.

Ensuring effective washing requires continuous monitoring of the system for factors that will influence washing (eg. sanitiser concentration, pH, contact time, temperature, organic content). If no monitoring is done, the washing procedure maybe of little benefit or even further contaminate the product (refer table 2). Garg et al. (1990) stated that inadequately monitored washing will initially clean produce earlier in the production shift but possibly contaminate product later in the shift. Hence it is important to monitor these critical parameters.

The recycling of wash water may be done by using filters and screens. This will conserve water, while reducing the volume of waste accumulation in the wash water.

Dewatering following washing of the product is useful for reducing the product moisture content and removal of cell leakage that can support microbial growth. Dewatering systems include spin dryers (centrifugal), vibrating racks, rotating conveyors, hydro-sieves, spinless dewatering and spinless drying tunnels.

The important elements of the washing system are displayed in Figure 2, a flow diagram depicting the pathway of the product, water recycling, the monitoring and dispensing of the washing agent.

Figure 1. Production of Minimally Processed Vegetables

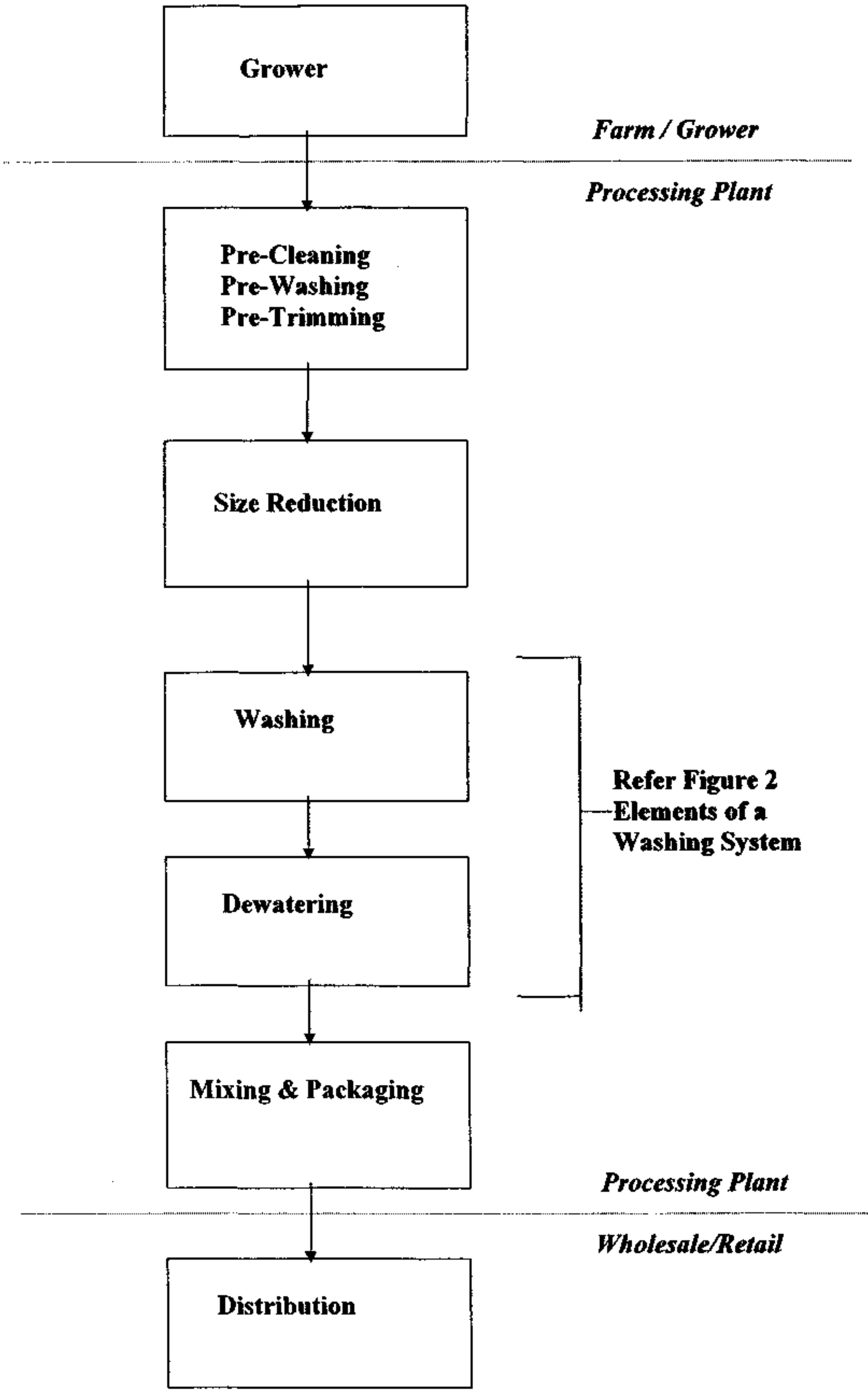


Figure 2. Elements of a Continuous Washing System.

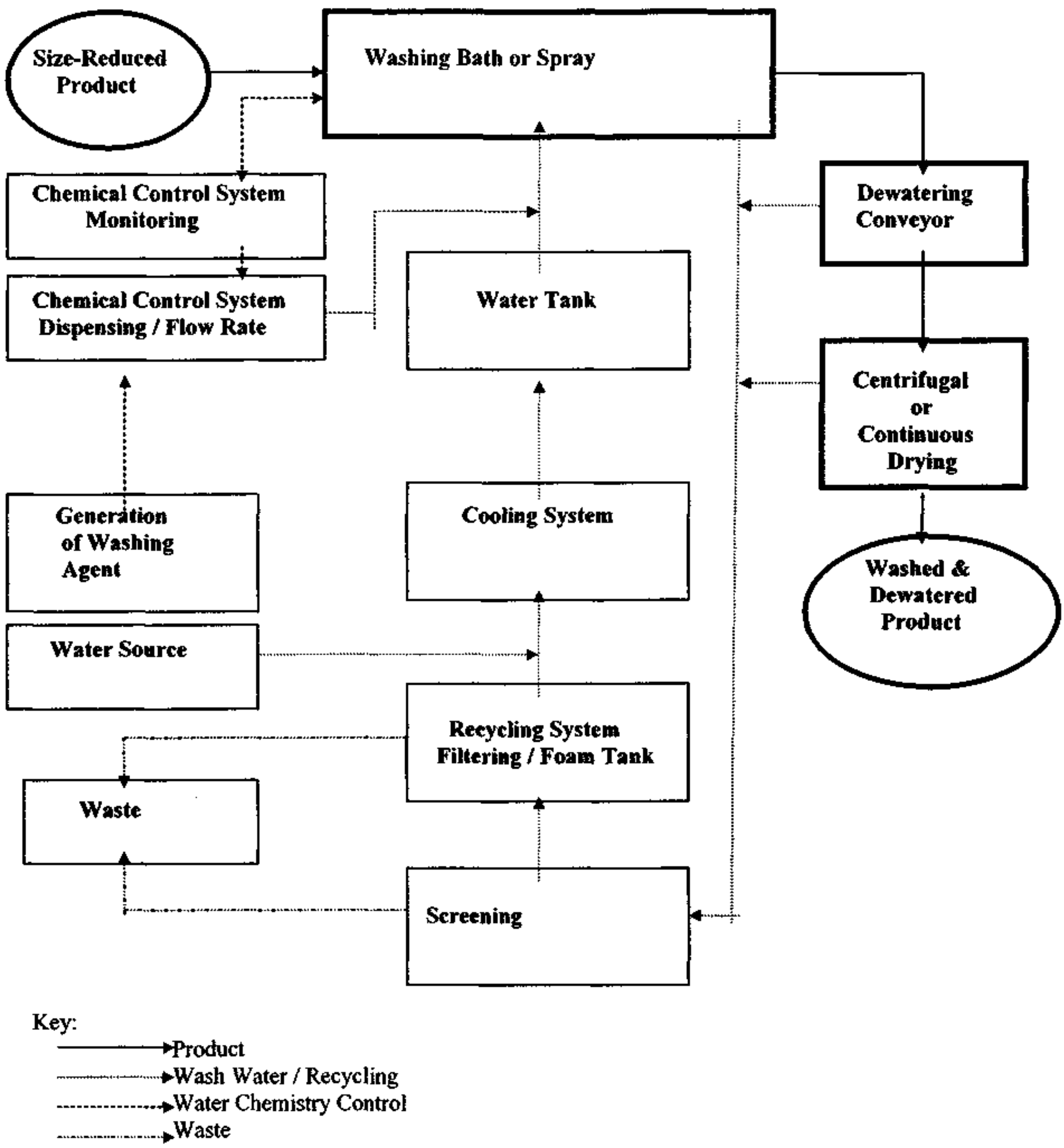


Table 1. Micro-organisms of Minimally Processed Vegetables
(Adapted from Nguyen-the and Carlin, 1994)

Pathogens from Vegetables reportedly linked to Foodborne Infections
Listeria monocytogenes
Escherichia coli
Salmonella spp.
Clostridium botulinum.
Vibrio cholerae
Shigella sonnei
Virus hepatitis A
Bacillus cerus

Other Pathogens of concern.
Aeromonas hydrophila
Yersinia enterocolitica
Campylobacter spp.
Staphylococcus aureus

Spoilage Organisms of Minimally Processed Vegetables
Pseudomonas fluorescens
Erwinia caratovora
Leuconostoc spp

2. WASH WATER

Traditionally, vegetable washing systems used chlorinated washing-water. Alternatives (or additions) to these systems include potentiators, shelf-life extenders, ozone, chlorine dioxide and post-wash dipping solutions. These wash-water systems are discussed with respect to their application and effectiveness for vegetable washing. Factors influencing the efficiency of washing are discussed with respect to reducing microbial load and physiological disorders.

2.1 Chlorinated washing water

Vegetables are commonly washed in chlorinated water prior to processing to reduce the microbial load. When elemental chlorine or hypochlorites are added to water, they undergo the following reactions:

$$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-$$

$$Ca(OCl)_2 + H_2O \rightarrow Ca^{2+} + H_2O + 2\ OCl^-$$

$$Ca(OCl)_2 + 2H_2O \rightarrow Ca(OH)_2 + 2\ HOCl$$

$$HOCl \Leftrightarrow H^+ + OCl^-$$

The term "free available chlorine" refers to elemental chlorine (Cl_2), hypochlorous acid (HOCl) and hypochlorite ion (OCl^-). The dissociation of HOCl depends on pH, and the equilibrium between HOCl and OCl^-, which is maintained even when HOCl is constantly consumed through its anti-microbial activity (Beuchat, 1992).

Antimicrobial Activity of chlorine:
Chlorine is active against a wide spectrum of micro-organisms, including viruses, non-acid-fast vegetative bacteria, acid-fast bacilli, bacterial spores, fungi, algae, and protozoa, with bacterial spores being the most resistant form of microbial life (Trueman, 1971).

Mode of Action:
The mode of action through which hypochlorous acid (HOCl) exerts its lethal effect on micro-organisms has not been clearly defined. Lethality is attributed to chlorine combining with cell membrane proteins to form N-chloro compounds which in turn interfere with cell metabolism. The inhibition of enzymes sensitive to oxidation by chlorine also appears to be involved in the killing of micro-organisms (Beuchat, 1992).

Effective Antimicrobial Concentrations for Chlorine:
Low levels of available chlorine (1-6ppm) are required to kill viruses, algae, and non-acid fast vegetative bacteria. Much higher concentrations of sodium hypochlorite are used for disinfection (ie. 150-200ppm) depending on the amount of organic soiling and the types of micro-organisms to be killed. The use of about 60-80 ppm of chlorine for a minimum of 30 seconds was recommended for the inactivation of enteric pathogens which might be present on fruits and vegetables (Hobbs and Gilbert, 1978). Adams et al (1989) reported that, increasing the washing time from 5 to 30 minutes

did not decrease microbial numbers further. They also found that washing with water was comparable to washing with 100 ppm of free chlorine at pH 9.0. Where a reduction of 92.4% and 97.8% of lettuce microflora was achieved.

Brackett (1987) observed the antimicrobial effect of free chlorine (in a phosphate buffer pH=7 prepared from chlorine-demand free water, where total Cl = free Cl) on *Listeria monocytogenes*. He found that free chlorine concentrations < 50 ppm showed no antimicrobial effect but exposure to $\geq$ 50 ppm chlorine resulted in no viable cells being recovered for a contact time of $\geq$ 20 seconds at a population of about 10^8 CFU/g. Chlorine solutions were prepared from sodium hyporchlorite and household bleach. With brussel sprouts containing about 10^6 CFU/g of *Listeria monocytogenes,* dipping in 200 ppm chlorine solution for 10 seconds reduced the population to about 10^4 CFU/g, while dipping in water alone reduced the population to about 10^5 CFU/g. The effect of adding 10 brussel sprouts decreased the amount of free chlorine from 178.8 ppm to 169.3 ppm. This was not considered to be significant, but successive additions of the product may reduce the chlorine levels sufficiently to impair the antimicrobial effect and replacement of the chlorinated wash water may be required.

Beuchat and Brackett (1990) investigated the effect of chlorine treatment (200 to 250 ppm free chlorine) on the growth and survival of *Listeria monocytogenes for* whole and shredded lettuce leaves packaged under modified atmosphere. A chlorine wash for 10 seconds, preceded by a 1 minute wash in tap water, and followed by a 1 minute wash in sterile water, significantly lowered the population of *Listeria monocytogenes* by almost a factor of 10. Results suggested that some chlorine may have remained on the lettuce leaf and had a lethal or sub-lethal effect on *Listeria monocytogenes*. Following, 15 days (whole leaves) and 9 days (shredded lettuce) of MAP storage, the population of *Listeria monocytogenes* was comparable to that of non-chlorine treated lettuce. Beuchat and Bracket (1990) concluded that chlorine treatment, modified atmosphere (3% O_2 and 97% N_2) and shredding did not significantly influence the growth of *Listeria* monocytogenes.

The importance of maintaining adequate chlorine in washing water was highlighted by Senter et al (1985).They observed that the total plate counts and enterobacteriaceae increased on tomatoes washed in water containing an average of 114 ppm (range 90-140) chlorine, but decreased in water containing 226 ppm (range 120-280) chlorine. Washing in the early processing shift may clean the product, but contaminate them in the later shift. Particularly if the same water is recycled, it will accumulate debris and increase microbial populations (Brackett, 1992).

Garg, Churey, and Splittstoesser (1990) investigated the effect of chlorine washing (and other processing conditions) on the microflora of fresh-cut vegetables, and found that counts were not always reduced (see table 2). The efficiency of decontamination depends on the product. The factory strived to maintain 300 ppm free chlorine, but this was difficult because organic solubles react rapidly with the chlorine, while chlorine additions were carried out manually.

It has been suggested that 50-200 ppm free chlorine is necessary to destroy vegetative bacteria and fungal cells in commercial vegetable packing houses. However, many producers feel that the upper levels can cause adverse discolouration and leave off flavours in the processed product (Hurst, W. A. and G. A. Schuler, 1992).

Table 2. Effect of chlorinated ice water bath (about 300ppm) on the Aerobic Plate Count.

VEGETABLE	BEFORE	AFTER
Carrot	6.4×10^5	5.7×10^5
Red Cabbage	9.6×10^5	1.1×10^6
Lettuce	1.4×10^6	2.5×10^3

(Adapted from Garg, Churey, and Splittstoesser, 1990)

The ability of conventional water and hypochlorite washing to effectively remove bacteria is limited by their survival in protective hydrophobic pockets and folds in the leaf surface. This statement has been supported by electron microscopy (Adams et al, 1989). Lund (1983) suggested that contact with host tissue may inactivate hypochlorite, and generally the adhesion of *Listeria* to inert supports increases the resistance to sanitisers.

Further research is required to determine the effect of different produce, load sizes, and the frequency of loads on the availability of free chlorine

2.2 Factors influencing washing efficiency

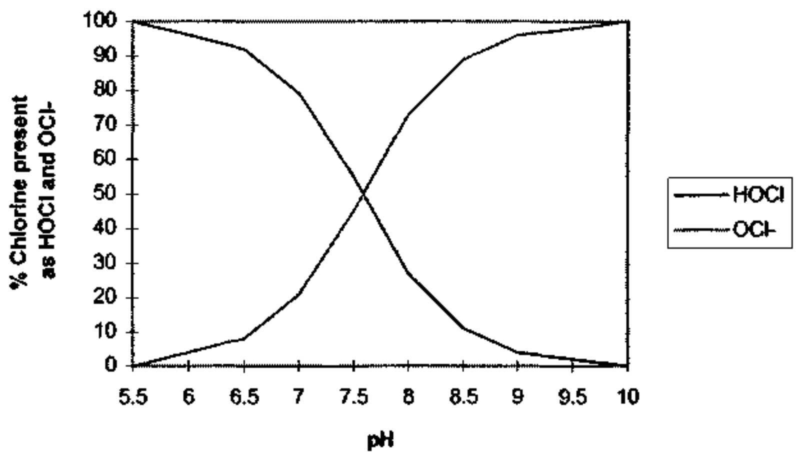
The relative proportions of the free chlorine forms (ie., aqueous molecular chlorine, hypochlorous acid, and hypochlorite ion) are pH and temperature dependent.

The availability of free available chlorine is dependent on
(i) pH
(ii) contact time
(iii) microbial population (in wash water & product, before and after)
(iv) condition of wash water (organic content)
(v) frequency of washing
(vi) temperature

Effect of pH:

Bacteriocidal activity of chlorine is associated with the concentration of undissociated molecules of hypochlorous acid. Since the dissociation of hypochlorous acid changes with pH, in theory maximum bacteriocidal effect should occur at pH 5. The minimum time required for a 99% spore death was observed at pH 4-5, with increasing death time required at higher pH. The pH is sometimes more important than the chlorine concentration. For example, 2 ppm available chlorine is lethal within 45 mins at pH 4 but ineffective after 2 hours at pH 5. It is equal in activity to 50 ppm at pH 9 and $\geq$ 100ppm at pH 10, for killing 99% of *B.anthracis* spores. (note: investigations were performed with spores because the death rates of vegetative cells were much to high to yield any comparable figures) (Sykes, 1965).

Figure 3. Effect of pH on Chlorine Dissociation Products



Adams et al., (1989) observed that an adjustment of pH from 9.0 to 4.5 with inorganic or organic acids produced a 1.5 to 4.0 fold increase in the microbiocidal effect for lettuce leaves. For chlorination to be effective, the recommended pH should be maintained at or near neutral (6.5-7.0). Above this OCl^- is formed, which is ineffective for disinfection purposes. While below neutral pH, $HOCl^-$ is formed which is highly effective for disinfectant purposes, but is extremely corrosive to equipment and can be very hard on vegetables (see figure 2).

Effect of Temperature:

The bacteriocidal activity of chlorine increases at higher temperature. For example the time for effective killing of Pseudomonads using 3ppm hypochlorite is 10 mins at 4.4°C and 4 mins at 21°C, giving a 35% increase in lethlal activity per 10°C rise in temperature (Hugo, 1971). However, washing water is generally cooled (eg. 4 °C) by water cooling systems or the addition of ice, to initiate or maintain the cold chain during processing. This is more benificial with repect to controlling microbial growth, reducing enzyme activities and product respiration.

Effect of Organic Matter:

The bacteriocidal activity of chlorine is reduced in the presence of organic matter and debris, as would be associated with the washing of vegetables. Chlorine does not generally react with carbohydrates, but will react with nitrogenous substances, forming *N*-chloro or a chloroamino derivative which retains some bacteriocidal activity, at a reduced level (Sykes, 1995). The accumulation of organic matter and debris in the wash water should be monitored, for when water replacement is required.

Toxicity:

The use of chlorine for water purification and washing presents health concerns particularly with respect to the formation of trihalomethanes (Menzer and Nelson, 1986). These include, chloroform, bromodichloromethane, dibromochloromethane, and bromoform which result from the chlorination of humic precursors. Raw-water of low turbidity yields fewer trihalomethanes than highly turbid water. Therefore it is possible that wash water contaminated with vegetable particles and juices will produce more trihalomethanes and chloroamines. Presently, it is generally accepted that there may be a relationship between rectal, colon and bladder cancer, and water quality. Also a significant association between breast cancer and chlorine levels in drinking water has been proposed (Menzer and Nelson, 1986).

2.3 Improvements by chlorine potentiation

The use of a potentiator can considerably improve the microbial quality of washed produce such as broccoli and cauliflower. In addition the potentiator can exhibit a residual microcidal effect for at least 15 days after packaging (Silvers, 1994), thereby extending shelf-life. Adding chlorine potentiator may reduce the required chlorine concentration by 30% to 50% without losing any sanitation effect. It may also extend the effectiveness of chlorine to a wider pH range of 5 to 10 rather than the 6 to 7 pH range for chlorine alone. Independent laboratory evaluation of the chlorine potentiator (from Bonagra Technologies & Services Inc.) demonstrated an effective increase in sanitation by a factor of at least 20, even after the reduction of the chlorine concentration from 50 to 100 ppm down to 35 to 50 ppm. The chlorine potentiator ($14-$16 per gallon) is mixed directly with sodium hypochlorite at a ratio of 1 to 4. The nature of potentiation and the active constituents was requested but this information was not provided.

2.4 Alternatives

Ozone:

The American food industry is currently evaluating the implementation of ozone into vegetable washing systems. This alternative was explored because the chlorine wash system can produce harmful disinfectant by products (eg. chloramines, trihalomethanes).

The antimicrobial effect of ozone is a result of its oxidising power which is lethal to a wide variety of microorganisms at ppm, and the oxidation of ethylene which retards the metabolic processes associated with ripening (Beuchat, 1992). Ozonated water (0.1-0.2 ppm) has been demonstrated to instantaneously kill 5 $\log_{10}$ units of *Salmonella typhimurium*, and *Escherichia coli*, 3 $\log_{10}$ units of *Pseudomonas aeroginosa* and *Yersinia enterocolitica*, 4.5 $\log_{10}$ unit of *Candida albicans Zygosaccharomyces bailli*, and 1 $\log_{10}$ unit of *Aspergillus niger* after five minutes (Restaino, 1995).

Clear Water Technology Inc. (1995), provides ozone generators for use in the washing process (from $20,000; 20 gram of ozone/hour). These were evaluated in an open flume washing system. Some of the advantages of an ozone system cited by *Clear Water Technology Inc* (1995), include the reuse and recirculation of wash water, reduced chemical use and costs, and reduction of corrosion. As with the chlorinated systems, it is important to ensure that particulates are filtered out to minimise available ozone depletion. Proper monitoring of ozone levels and water chemistry is also necessary to ensure effective sanitation. The *Clear Water Technology* system continuously monitors water flow and ozone levels via an in-line oxidation reduction potential (ORP) probes. In addition, the ozone system should also include an air ozone monitor to insure that ozone levels in the atmosphere do not exceed safety standards, since ozone can be harmful to human lungs. Independent factors that influence the overall effectiveness of ozone as a disinfectant are; (1) water temperature, (2) ozone concentration (3) non-target demand substances (4) specific surface area.

Independent research by the California Polytechnic State University using the *Clear Water* Ozone Technology showed that ozone (0.64-1.11 ppm) was a better disinfectant than Cl_2 (100 ppm). An additional 2 $\log_{10}$ reduction in the total plate count was achieved when ozone was compared with Cl_2. Broccoli-stems(1.11 ppm) required the lowest contact time, followed by broccoflower (1.08 ppm), and then carrots (0.64 ppm). This is contradictory to research by Wolfe et al.(1989) comparing the disinfection effectiveness of drinking water by ozone, chlorine, chloramine and peroxone (H_2O_2/O_3). The comparative disinfection experiment indicated that free chlorine was a more potent bactericidal agent, followed (in descending order of effectiveness) by ozone, peroxone and chloramines.

No comparison was made with chlorine systems that included a chlorine potentiator (eg. Bonagara Technologies & Services, Inc.). There was no information with respect to residual effect of ozone from shelf-life studies. There is more requirement for comparative studies with chlorinated systems. This could involve ozone combined with chlorine to determine if their is a synergistic effect.

Ozone gas and an aqueous ozone solution have been used in combination to sterilise fresh fruits and vegetables (Matsunaga et al., 1995). This involved washing the vegetables then subjecting to ozone gas at a concentration of 170 mg/L at 25°C for 20 minutes. The food was then peeled and immersed in an aqueous solution of ozone at a concentration of 20 mg/L at 20°C. After which the food was removed and dried. This process has a wider antimicrobial spectrum than treatment with ozone gas or solution alone. There is also another ozone washing method that has been developed for the washing of MPVs. The general method involves washing the cut produce in chilled-chlorinated water, drying and then rewashing in water in an overflow type air-bubbling tank, drying, and a final washing step using ozonated water (Daiee and Okishio, 1995).

Chlorine dioxide:

Chlorine dioxide is a powerful disinfectant and oxidising agent, and has been applied to the treatment of municipal and waste water. Other chlorine dioxide applications include the sterilisation of fluming, can-cooling, washing, wash-down recirculated or discharge water; and control of taste and odour in the process water used in soft drink bottling, brewing and distilling.

Chlorine dioxide is unstable and must be generated on-site. *Wallace & Tiernan* supply chlorine dioxide generating systems (from $35-38,000) in which sodium chlorite reacts with chlorine to from chlorine dioxide and sodium chloride. Using the appropriate substrate ratio, nearly pure chlorine dioxide can be produced. For efficient conversion of sodium chlorite to chlorine dioxide, a high concentration, low pH chlorine solution is required. The *Wallace & Tiernan* system is advantageous because it utilises continuous batching and optimum hydraulic conditions to maintain the pH at a low level with out the requirement to add acid or excess chlorine. A metering pump (DE-60) is used to discharge chlorine dioxide solution to the point of application. The system can be controlled manually, but also accepts, remote and automatic control from electronic pulse frequency control systems, ratio controllers or set value controllers.

$$2\,NaClO_2 + Cl_2 \rightarrow 2\,ClO_2 + 2\,NaCl$$

Unlike chlorine, chlorine dioxide does not hydrolyze in water to form hypochlorous acid (free available chlorine). Instead, it remains dissolved as a gas and may decompose to its chlorite and chlorate ionic forms. Chlorine dioxide dissolves the microbial cell wall, hence its kill time is much shorter than liquid chlorine (Hurst, 1995). It does not react with ammonia to form chloramines and trihalomethanes and is effective in waters with high organic levels, and is essentially unaffected by pH. These characteristics make chlorine dioxide a favourable alternative to chlorine.

Chlorine dioxide (50 ppm) washing of mushrooms was found to be effective in controlling post harvest bacterial growth while retaining colour during storage (Guthrie and Beelman, 1989). A dipping solution containing 0.1-0.15 g/L chlorine dioxide reduced the incidence of soft rot in potatoes of mainly *Erwinia caratovora ssp caratovora* and pectolytic Clostridia (Wyatt GM and Lund BM, 1981).

The *Wallace & Tiernan* chlorine dioxide system is used at two vegetable processing plants in Australia, *Simplot (previously Edgells)* in Devonport and *Edgells* in Towoomba, for the washing of vegetables and supply of potable water. The Devonport plant washes carrots, broccolli and potatoes in ClO_2 (up to 3ppm), for canning and freezing. To our knowledge ClO_2 is not used in Australia for the washing of minimally processed refrigerated vegetables.

No published literature was found on the use of chlorine dioxide wash water on minimally processed lettuce, broccoli or carrots. Therefore, research is required to determine the effectiveness of chlorine dioxide with respect to the washing of popular minimally processed vegetables. A comparison should be made with chlorinated and ozone washing systems, this could involve an industry survey, which may have to include vegetable processors other than strictly minimal processors (ie. canning and freezing) to encompass all potential washing technologies.

2.5 Post-wash dipping solutions

The shelf-life of minimally processed vegetables (lettuce, broccoli and carrot) can be limited by the development of physiological disorders, giving an unacceptable appearence to the consumer. Post-wash dips and coatings can be used to supress physiological disorders.

The shelf-life of minimally processed **lettuce** can be limited by browning. This results from both enzymatic polyphenol oxidase (PPO) and non-enzymatic oxidation of phenolic compounds. Vegetable phenolic substrates include, caffeic acid, protocatechuic acid, chlorogenic acid and tyrosine. The initial products of oxidation are quinones, which rapidly condense to produce relatively insoluble brown polymers (eg. melanin) (Martinez and Whitaker, 1995). Browning usually impairs the sensory properties of products because of the associated changes in colour, flavour and texture (due probably to the action of pectic enzymes). Once cell walls and cellular membranes lose their integrity, enzymatic oxidation proceeds much more rapidly (Martinez and Whitaker, 1995). The enzyme phenylalanine ammonia lyase (PAL) is important in the biosynthesis of phenolic substrates for PPO. When minimally processed lettuce was treated with ethylene the PAL and PPO activities increased in correlation with the browning intensity (Couture et al., 1993).

The shelf-life of minimally processed **carrot** (especially abrasively-peeled carrot) can be limited by whitening. The whitening may be due to the formation of lignin as a wound barrier (Bolin and Huxsoll, 1991; Howard and Griffin, 1993), and the dehydration of abraded surfaces (Tasumi et al., 1993). The surface discolouration of carrot battons/sticks can be limited by steam treatment (Howard et al., 1994), sodium chloride treatment (Tatsumi et al., 1991), dipping in heated acid or alkali solutions (Bolin and Huxsoll, 1991), or by the application of edible coatings (Breummer, 1987).

The shelf-life of minimally processed **broccoli florets** can be limited by yellowing of the flowerheads (Brackett, 1989). In addition to whitening and browning of cut surfaces.

Control of physiological disorders:

Enzymic browning requires four essential components; (i) oxygen, (ii) enzyme - phenol oxidase, (iii) substrate,(iv) copper, with correct pH and temperature conditions. To control browning, one or more of the essential components must be eliminated from the reaction. Traditionally, sulphite has been used as an inhibitor of browning reactions in foods. Potential alternatives to sulphite include (Lyengar and McEvily, 1992):

(1) Enzyme Inhibitors: (eg. aromatic carboxylic acids, aliphatic alcohols, substituted resorcinols, anions, peptides)

(2) Reducing Agents: (eg. sulfiting agents, ascorbic acid and analogues, glutathione, cysteine)

(3) Enzyme Treatments: (eg. oxygenases, O-methyltransferases, proteases)
(4) Chelating Agents: (eg. phosphates, EDTA, organic acids)
(5) Acidulants: (eg. citric acid, phosphoric acid)
(6) Complexing Agents: (eg. cyclodextrins, chitosans)
(Lozano-De-Gonzalez et al., 1993).

An effective sulfite substitute to prevent browning reactions involves a combination of ascorbic and citric acid. For short protection times (2-4 hours at ambient temperature) freshly prepared vegetables are recommended for immersing in a 1-2% citric acid bath for 30-35 seconds. For 4-7 days shelf-life, the bath should contain 1% citric acid + 0.5-1.0% ascorbic acid for 30-45 seconds (Anon, 1983).

Commercially available anti-browning solutions typically contain a reducing agent (eg. ascorbic acid), an acidulant (eg. citric acid) an enzyme inhibitor (eg. calcium chloride) and a chelating agent (eg. sodium acid pyrophosphate) (Beck, 1992; Dziezak, 1988; Warren, 1991). Aqueous wash solutions containing an anion such as phosphate, pyrophosphate, sulphate or chloride to inhibit discolouration are maintained at pH less than 2, for a sufficient time to arrest the phenolase colouration (Schwank, 1992). Commercial dips/sprays are available for broccoli (to retard yellowing), carrot (to retard white-blush) and lettuce (to retard browning) (Kravitz, 1994). Vege-fresh and sodium dehydroacetate were found to substantially reduce the amount of discolouration (browning) of shredded lettuce (Hicks and Hall, 1972). These washes achieved a shelf-life of 15 days compared to about 6 days for water washed controls stored at 3°C. The formula and the time of application can be varied to achieve a longer shelf-life (Kravitz, 1994) (refer, table 3). A list of commercially available anti-browning agents and their ingredients has been compiled by Kim and Taub (1988).

Post-wash dips also include the application of edible coatings. Basically there are three groups of materials used in coatings, namely proteins, carbohydrates, and lipids, including waxes, and emulsifiers. Coatings could potentially contain a combination of the following features; (1) forming an efficient barrier to moisture loss, (2) a selective permeability to gases, (3) controls migration of water-soluble solutes to retain the natural colour pigments and nutrients, and (4) may incorporate additives such as coloring, flavour, or preservatives that impart special functions and properties(Wong et al., 1994).

Coatings have been successfully applied to abrasively-peeled carrots to reduce white-blush (Avena-Bustillos et al, 1993; Reyes et al., 1996), and to reduce moisture loss from celery sticks (Avena-Bustillos et al, 1994). Both these coatings were a caseinate / acetylated monoglyceride / alginate mixture. In most cases ascorbate solutions containing calcium are used, both for the purpose of cross-linking and for preventing the cut surface from browning. Calcium is used primarily to prevent loss of firmness, which is attributed to the complexation between calcium and pectic acid. It also serves as a binding point for protein-protein complexes, which is important for maintaining cell-to-cell adhesion. Calcium may inhibit the activity of cell wall-degrading enzymes. The cross-linking interaction with pectic polymers may also render the substrate inaccessible to enzyme action. Calcium was found to supress the respiratory activity and ethylene production. May also be involved in the regulatory functions of many enzymes (Wong et al., 1994).

With the extension of the physiological shelf-life (refer Table 2) achievable when using post-washing dips and coatings, the growth of microbes becomes a concern. In particular psychrotrophic strains of *Listeria monocytogenes, Yersinia enterocolitica* and *Aeromonas hydrophila* that can grow at refrigeration temperatures (Palumbo, 1986). Spoilage bacteria, notably *Pseudomonas sp* can grow relatively rapidly under refrigeration temperatures. Many strains have pectinolytic enzymes which can degrade fresh product (Brackett, 1992). This highlights the importance of a washing agent that has a residual antimicrobial effect, such as the chlorine potentiator (*Bonagara Technologies and Services, Inc*).

Table 3. Effect of washing treatments on the shelf-life of broccoli and carrots

Treatment	°C	Shelf-life	Limiting Factor	Source
Carrot (abrasively peeled):				
Water wash	3.3	1 day	White-blush	Extended Product Life, Inc.
Post-wash dip in *Carrot Fresh*	3.3	21-28 days	White-blush	Extended Product Life, Inc.
Broccoli Florets:				
Chlorine wash	3.3	15 days	Yellowing	Ballantyne et al., 1988.
Post-wash spraying with *Broccoli Fresh*	3.3-4.4	31 days	Yellowing	Extended Product Life, Inc.

Antimicrobials (preservatives) that maybe incorporated into edible coatings, include benzoic acid, sodium benzoate, sorbic acid, potassium sorbate, propionic acid and chitosan. Coatings can help hold the preservative on the cut surface where it is needed. The combination of acid and coating can result in improved microbial stability of the product (Baldwin, et al., 1995; Reyes, et al., 1995).

2.6 Monitoring washing water

Chlorine determination: (for free chlorine)

Iodometric methods are suitable for measuring total chlorine concentrations > 1ppm, however the estimation of free-chlorine (eg. Cl_2, HOCl and OCl^-) is more appropriate as it posses the major antimicrobial activity.

The American Public Health Association, standard methods for the examination of water and waste water, describes three methods for the determination of free-chlorine. (1) Amperometric titration method, (2) *N,N*-diethyl-*p*phenylenediamine (DPD) Ferrous Titrimetric Method, (3) DPD Colorimetric method.

Sample colour and turbidity may interfere with the colorimetric methods, and they are unlikely to be suitable for wash baths with vegetable debris. In contrast, the amperometric titration method is not overly affected by common oxidising agents, temperature variations, turbidity, and colour. However, this method is not as simple as the colorimetric methods and requires greater operator skill to obtain the best reliability.

Online Monitoring:

All commonly used test kits and "automated test kits" see free oxidant as a single value which is the sum of hypochlorous acid (HOCl) and hypochlorite species (OCl^-). They cannot discern the degree of dissociation occuring in the system. The impact of this deficiency is dramatic because hypochlorous acid is much more effective, but the presence is critically dependent on pH. For example at an operating pH of 8 an increase in pH of 0.5 decreases HOCl by 59%, while an equivalent decrease in pH increases HOCl by 109%, thereby changing the oxidative work value.

The High Resolution Redox (HRR) is the only available instrumentation which provides long-term, unattended, on-line control of both chlorination and dechlorination (*Stranco)*. This system recognises changes in the oxidative work value and reponds to fluctuations in demand.

The *Stranco* system for monitoring wash water, uses two specially designed probes with automatic dispensing control to maintain chlorine ($\pm$ 0.1 ppm) and pH ($\pm$ 0.1) at precise levels. The importance of effective chlorine and pH control in washing systems was demonstrated in onion washing where the shelf-life was extended from 5-7 days to 10-14 days. The shelf life extension was also attributed to the use of a flood washer as opposed to a spray washer (Anonymous, 1995).

Dispensing pump types: (*Wallace & Tiernan*)
(1) manual continuous with disinfectant feed rate adjustment
(2) auto-proportional control with disinfectant feed rate based on flow rate
(3) compound loop control disinfectant feed rate based on flow rate and
chlorine demand, indicates deviation from setting point
Available for chlorine, chlorine dioxide, ozone, and pH control.

Microbiology:

For the evaluation of washing performance it is important to monitor microbial population. This should be conducted before and after washing to determine if a sufficient microbial kill has occurred. In addition, monitoring changes in microbial population during the shelf-life study would be useful to determine if any residual antimicrobial effect occurs with particular washing agents and to provide shelf-life estimations.

Impedence technology could be used for the rapid evaluation of the effect of disinfectant, preservatives and organic matter on bacterial growth (Siley, 1991).

Impedednce technology has been used for the rapid detection of microbial contamination in vegetables (Hardy et al., 1977). This method requires the calibration of the impedence threshold detection times (TTD) with traditional culture methods. The advantage with the impedence technology is that it is rapid and automatic. Bacterial populations can be estimated in approaximately 10 hours, where as traditional plating methods may take up to three days. However the instrumentation required is expensive.

The new 3M™ series 200 Plate has been designed for the rapid detection of Coliforms. Instead of waiting 24 hours for coliform counts, presumptive colonies may appear at 6 hours of incubation and confirmed colonies at 8 hours. High levels of coliform contamination may be apparent after only 4 hours. This method offers the additional advantages of being cheap, lowering labour costs, allowing quality assurance teams to read results will before the product is further processed or released (3M™ Microbiology Products, 1995).

The microbial analysis of the wash water can be performed using a membrane filtration method, this is particularly practical for larger volumes of water with low microbial numbers.
The methodology is outlined in the Australian Standards 1766.1.5 (1991). Bacteria trapped on the membrane filters which are then be incubated on agar plates, or wetted with culture media. Membran filtration is useful because it will allow the washing agent to pass through, while trapping the bacteria which avoids any residual (antimicrobial) effect from the washing agents. Where as, if the washing agent was not removed its activity would have to be neutralized.

If the testing for pathogens is required this can be performed using a number of rapid methods, including the indirect-impedence method (using selective media), immunoassays kits which include enzyme linked immunoadsorbent assays (ELISA) or imuno-magnetic beads and DNA/RNA probes utilizing Polymerize Chain Reaction (PCR).

There is a requirement to develop measuring protocols for determining the effectiveness and contamination of washing systems, including fast detection methods for microbial populations. In this respect, here is a link to CRC program 5.2.1 *Rapid Detection Technologies for the assessment of food preservation and microbial saftey.*

3. SURVEY OF EQUIPMENT AND SUPPLIERS

Information regarding washing, dewatering and recycling systems for minimally processed vegetables (MPVs) was obtained from journal articles and patents sourced by CD-ROM searches of the Food Science Technology Abstracts (FSTA), CAB Agricultural Abstracts and Horticultural Abstracts. Relevant equipment suppliers were sourced from the Food Industry Sourcebook (1995), Produce Processing Equipment Buyers Guide (1994), the Food Processors Guide (1987) and from internal AFISC industry knowledge.

Equipment suppliers were requested by fax to provide information on washing and dewatering systems, washing chemicals, monitoring and dispensing units, water recycling systems, and any equipment evaluation report or performance test data. A total of 34 companies were contacted in this way requesting relevant information, of which 15 responded (refer 6. Equipment Suppliers List). Equipment types, design and effectiveness are discussed for washing, dewatering and recycling systems in the following sections, 3.1, 3.2 and 3.3 respectively.

3.1 Washing systems

Types of washing systems include (i) closed flume (ii) open flume and (iii) wash tanks. The choice of system is dependent on the products being washed. The shorter wider systems, have lower velocity while providing a gentler wash (see table 4). While the closed pipe systems, have a much higher velocity which can damage delicate produce such as broccoli and cauliflower florets.

The closed flume systems are advantageous with respect to cost, simplicity, low floor space requirements, good product immersion and ease with which food can be elevated. While, the open systems are favoured for leafy vegetables and florets, which require a gentler wash in comparison to carrots.

The rod-reel washer and the scrubbing-peeling washers were designed especially for carrots (and other tubers). Broccoli and lettuce should receive a gentle washing action as not to physically damage the tissue, causing the release of cell nutrients that will support microbial growth and reduce visual acceptance. Gentle washing systems include wide flumes or wash tanks. Systems that ensure mixing of the wash water will improve washing effectiveness. This is improved in some designs which include air jets, water jets and counter-flow. With some designs, the washing intensity can be varied by carrying out two or three washing steps Alternatively, the washing retention time can be prolonged by the use of specially designed aeration valve systems, varying the conveyor speed or extending the length of a flume. These attributes allow for the optimisation of the washing system with respect to sanitiser contact time.

Washing of fresh vegetables by supersonic oscillation was investigated with respect to the removal of Cu and Cu-containing pesticides. Supersonic washing removed 60% of Cu from lettuce leaf compared with 30% when washed. Prolonged exposure of lettuce leaf to supersonic waves may disintegrate tissue cells (Kimura, 1969)

Table 4. Characteristics of Flume-washing systems

WASHING SYSTEM	DIMENSIONS (width, length)	PRODUCE VELOCITY	WASHING TIME
Closed Flume	15 cm ∅ pipe, 50 m long.	1.2-1.5 m/sec.	37.5 secs
Open Flume	30cm wide, 15 m long.	0.3-0.6 m/sec	33 secs
Wash Tank	90 cm wide, 5 m long.	0.3 m/sec	30 secs

Source: Adapted from Schneider (1994)

Table 5. Washing Systems:

Type	Description	Produce / Application	Supplier
Flood Washers	combined flotation and overhead rinsing	leafy vegetables 20 t/hr, high volume	A.K.Robins
	counter flow, aerated water, recirculating disinfection system	leafy vegetables 1-6 t/hr	Backus Sormac BV
	flotation washer	broccoli, spinach, peas, beans 3 t/hr	Tripax Engineering
	compact low volume	beans, carrots 0.2-0.7 t/hr	Lyco Manufacturing
	food pump 4" gives prewash, riffle pan, separation tank, recirculation	peas, dry beans	Key Technologies
Froth Flotation Washer	aerated water	peas, beans, 1.3 t/hr	A.K.Robins, G. J. Olney
Rod-Reel Washer	rotating cylinder partly immersed in water bath	carrot, peas, beans	A.K.Robins
	rotating cylinder partly immersed in water bath	carrot, peas, beans 2-10 t/hr	Tripax Engineering
Washing-Scrubber-Peeler	abrasive peeling, and washing; continuous flow through or batch; separation of waste. .	carrots 0.9 t/hr	Magnuson Corp.
	continuous abrasive peeler	carrot, potato, turnip, 2 t/hr	Tripax Engineering
Brush Type Washer	brushes to clean submerged produce	carrot, potato, turnip, 2-5 t/hr	Tripax Engineering
Open Flume	optional water jets, sand traps, water chilling, recycling, transition slide coating to prevent product damage	lettuce, carrot , broccoli Dwell Time 30 secs.	Heinzen Manufacturing, Inc.
Spray Washing Conveyor	high capacity flowing water upon conveyor with screen filtration, and chilling system to	lettuce, carrots, celery, capsicum, etc. 0.5-1.4 t/hr	Freshline Machines P/L Aust.

	0.5 °C		
Rotary Counter Flow Cooler	immersed in cooling water, on conveyer in opposing direction to water flow	diced carrot, peas 10 t/hr, 4 min. cooling time	Tripax Engineering
Continuous Bucket Cooler	immersed in cooling water	broccoli 1-2 t/hr, peas 2-5 t/hr 2 min. cooling time	Tripax Engineering
Spray Cooler	spray cool water upon wire mesh conveyor	broccoli, carrot, spinach, etc.	Tripax Engineering

3.2 Dewatering systems

Dewatering systems generally include the perforated shaking trays, of which the double deck design is likely to more effective by being able to turn the product over. These may preclude the delivery of the produce into a centrifugal (spin) dryer. These are very practical if they can be added to a processing line as single units increasing flexibility, rather than in-line. Spin dryers vary from small fixed-baskets, to a much larger lift out baskets (Casquilho, 1993). The bottom dump basket eliminates the requirement for an overhead crane. Improved spin dryer designs include self balancing, braking and accurate speed control. By their nature spin dryers work in batches and are labour intensive. Continuous drying systems that operate in-line can reduce manual labour while, avoid a dewatering bottleneck, and achieve higher through-put. The *Bock Engineered Products* manufacture the Tornado continuous system, which consists of rotating conveyors. The system slows down for the simultaneous input of wet produce and output of dry produce. The *Backus Sormac* continuous system is a dewatering conveyor that turns over the produce, which can remove approximately 90% of the moisture without product damage. The remaining moisture can be removed by a drying tunnel to achieve even moisture removal with none of the physical damage (bruising) that can occur from centrifuges.

Table 6. Dewatering Systems:

Type	Description	Product / Application	Supplier
Dewatering Shaker	perforated shaking tray with water catch pan, interchangeable screens	carrots	A. K. Robins
	double deck, to turn product over, discharge chute to spin dryer	lettuce	Heinzen Manufacturing, Inc

	vibratory hydro-sieve	carrots	Tripax Engineering
Centrifugal Spin Dryers	fixed basket, self balancing, remove product by hand	lettuce 14-45 kg/hr	Bock Engineered Products, Inc
	lift out basket by crane	lettuce 60-900 kg/hr	Bock Engineered Products, Inc
	lift out basket by crane	lettuce 68-135 kg/hr	Heinzen Manufacturing, Inc
	bottom dump basket, 20 sec. dynamic braking	23-135 kg/hr	Heinzen Manufacturing, Inc
	electronic braking and speed control	10 kg in 100 secs. lettuce, broccoli, carrots	Freshline Machines P/L Aust.
	lift out basket	lettuce	Tripax Engineering
Drum Dryer	double-drum rotating screens	90 kg/hr	Lyco Manufacturing
Continuous Drying Systems	rapidly rotating conveyors	lettuce 1800-2700 kg/hr	Bock Engineered Products, Inc
	spinless dewatering, turn over on conveyor	lettuce	Backus Sormac BV
	spinless drying, conveyor through drying tunnel	lettuce	Backus Sormac BV

3.3 Cleaning wash waster / recycling

Most washing systems lack efficient water-recycling to a point where the wash water becomes increasingly saturated with vegetable, and particle juice and has to be dumped and replaced.

Water from washing and dewatering systems may be recycled using a hydro-sieve, dewatering conveyor, or a reclaim reel. Each are only effective in removing product particles that might otherwise clog up the water chiller or spray nozzles. However, they are not effective in removing the vegetable juices that will reduce the sanitising performance and support microbial growth. Filters may be used following screening to remove much smaller particles. The system may consist of a series of screens and filters as described by Schneider (1994_b). The wash-water used in the *Scalime* washing system for lettuces is tripled filtered to 50 microns for the initial spray and water bath, and filtered to 10 microns for the final water bath step (Robson, 1995).

Improvements upon the above designs include foam tanks (Heinzen Manufacturing, Inc.) which generate foam to carry off water soluble materials. The foam is then discharged before the water goes back to the chiller. This effectively reduces the biological and chemical oxygen demand of the water and minimises water usage.

The continuous deflective seperation unit designed to trap storm water debris could be applied to wash water recycling (Humphries, 1995). This system might be used for initial screening followed by finer filtrator(s) or a foam tank. The advantage of this system is that it can handle large volumes, is none clogging, cheap, low maintenance, and easy litter removal.

Ozone is useful for the cleaning of recycled wash water, because it has a biocidal activity over a wide pH range. Filtering prior to ozone treatment, avoids unnecessary ozone depletion (Silvers, 1994a). Effective disinfection at ozone concentrations of 0.5ppm and higher (Silvers, 1994b). In adddition to its sanitsing effect, ozone is a powerful oxidizing agent that will decompose and coagulate organic matter in processing waters. Which is an advantage over chlorine dioxide with respect to improving water clarity.

Ultraviolet light is a cheap non-chemical alternative to the sanitisers previously mentioned. The peak region of germicidal effectiveness in 240-260nm. The lethal effect is the destruction of the micro-organism's reproductive system. This is an extremely rapid method which involves, one or more germicidal lamps mounted axially in a treatment chamber and protected from the water by quartz sleeves. Water is disinfected in 1-2 seconds as it flows over the sleeves. The *Backus Sormac B. V.*, wash water recycling system uses ultraviolet light to kill bacteria, thus reducing chlorine usage (Anonymous, 1995_a). Organic matter in the washing water absorbs ultraviolet light which diminishes the effectiveness. Therefore the water should be filtered to reduce orgranic levels prior to ultraviolet treatment. Some specially designed systems include lamps with wipers that glide over the lamp sleeves to remove deposits that may block the ultraviolet light (Hurst, 1995).

For the recycling of washing water ozone is superior for improving water clarity. This could be best implemented to decompose and coagulate organic matter prior to filtration. If additional sanitising is required this could be followed by ultraviolet treatment of the clarified water.

Table 7. Recycling systems:

Type	Description	Supplier
Filtration / Foam tank / Chiller	Wash water from flume first filtered, foam generated in foam tank to carry off water soluble materials and particulates, then cooling, prior to returning to the flume.	Heinzen Manufacturing, Inc
Screen Filtration / Chiller	Chills water (off-peak) to 0.5 °C, minimises water usage, combined with spray washer, 1440 L chiller storage tank, continuous automatic system	Freshline Machines P/L Aust.
Stationary Screen Sieve	Initial screening of waste water vibratory hydro-sieve	Lyco Tripax Engineering

Water Reclaim Reel	High volume, worm-screw effluent removal	A. K. Robins,
Continuous Deflective Separation Unit	Designed to trap storm water debris (leaves, grass 99-100%), in a perforated basket. Potential for recycling of wash water, cheap, flexible, low maintenance, easy litter removal	Pollutec Water-Pollution Control
Ultraviolet	Use of ultraviolet rays to kill microflora, in conjunction with chlorine.	Backus Sormac BV

4. CONCLUSIONS

4.1 Wash Water-Sanitising Agent

The best washing-dewatering system must provide a product that retains physiological quality, while effectively reducing the microbial load to achieve prolonged shelf-life.

The continuous monitoring and dispensing of the sanitizing agent is essential, along with other important measurements that influence washing effectiveness (eg. pH, temperature). These control systems are currently available for chlorine, ozone and chlorine dioxide. An ideal system should account for variation in the load frequency, organic water content, surface area of the food, and the decay rate of ozone and chlorine dioxide, thereby ensuring an effective concentration of the washing agent is always maintained.

With respect to the sanitizing agent there is the choice of remaining with the traditional chlorinated system that may be considerably enhanced by the use of chlorine-potentiators, or investigating the use of the alternatives, namely ozone and chlorine dioxide. Both these systems may potentially provide advantages over the traditional chlorinated system (see table 8).

The pilot-scale research into ozone treatment suggests that it is more effective than chlorine, however, no comparison was made with chlorine systems that include a chlorine potentiator (eg. Bonagara Technologies & Services, Inc.). The potentiated chlorine wash provides a residual antimicrobial effect which extends microbial shelf-life and safety. However, with respect to ozone there was no information on residual effect obtainable from shelf-life studies. There is a requirement for comparative studies with chlorinated systems, which could involve ozone combined with chlorine to determine if their is a synergistic effect.

There is a lack of research into the suitability of chlorine dioxide in washing systems for minimally processed vegetables, while potential advantages warrant such research (see table 8). This is supported by its suitability in the treatment of municipal and waste water treatment; sterilisation of fluming, can-cooling, wash, wash-down recirculated or discharge water; and to control taste and odour in the process water used in soft drink bottling, brewing and distilling. If sanitising is the main priority than chlorine dioxide is the most promising washing agent.

The advantages of the potentiated-chlorine, ozone and chlorine dioxide systems would be limited if the quality of the wash water cannot be retained. This emphasises the requirement for an effective recycling system.

Table 8. Comparison of Sanitiser Washing Systems

Washing Factors	Chlorine	Potentiated Chlorine	Ozone	Chlorine Dioxide
Effective concentration	50-100 ppm	35-50 ppm[a]	1 ppm	1-3 ppm
Effective pH range	Critical control required range of 6.5-7.0	Wider pH range but optimum of 6.5	Relatively unnaffected at pH 6.0-8.0	Broad effective pH range
Effect of organic content [b]	Binds to organics	Binds to organics	Decompose & coagulate organics	Minimal reaction with organics
Residual effect	None	2 weeks	Unknown[d]	Unknown[d]
Toxicty[c]	Chloramines[b] Trihalomethanes Chlorine: TLV = 1 ppm TDL = 15 ppm	Same as for chlorine but maybe reduced chloro-organic levels.	Ozone (gaseous) release must be monitored, TLV = 0.1 ppm TDL = 2 ppm	ClO_2: TLV = 0.1ppm TDL = 19 ppm
Stability	Stable	Stable	Unstable, must be generated on-site	Unstable, must be generated on-site

([a]in a potentiated chlorine wash system 30-50% less chlorine is required)
([b]reaction with organic compounds reduces availability of washing agent and can form toxic by-products)
([c]TDL = threshold limit value, the concentration of an airborne constituent to which workers maybe repeatedly exposed day by day without adverse effect. TDL = toxic dose level, with respect to inhalation. (International Technical Information Institute, 1985))
([d]not likely to be a residual antimicrobial effect, due to their unstable nature)

4.2 Washing Equipment Design

The washing system should be selected to achieve effective sanitiser contact and minimise physical damage particularly with respect to lettuce and broccoli. The more versatile systems allow for the variation in washing time and intensity. All these characteristics were offered by the *Backus Sormac* flood washer and dewatering system which minimises physical damage.

The continuous dewatering systems including the (*Bock Engineered Products, Inc.*) rotating conveyors and (*Backus Sormac*) continuous dewatering conveyor offer considerable advantages over the other batch centrifugation (spin) dryers, with respect to throughput and reduced manual labour. However, the spin dryers are cheaper and more flexible. The (*Backus Sormac*) continuous dewatering conveyor followed by a drying tunnel is advantageous by providing a more even and thorough drying, without physically damaging the product, which can occur with centrifuges. However, the elevated temperature may temporarily enhance the growth of micro-organisms and increase product respiration. No performance testing data was supplied to evaluate this treatment on microbial and physiological shelf-life.

4.3 Post Wash Dips

The effectiveness of a washing system can be further enhanced by the inclusion of post-wash dips specifically designed to reduce physiological disorders (eg. yellowing of broccoli, whitening of carrots, browning of lettuce). These agents need to be prepared for individual produce, and maybe incorporated in the wash water or used in an additional washing step. Scope exists for improving the effectiveness of these dips, particularly with respect to controlling microbial growth as well as physiological disorders.

4.4 Recycling Washing Water

For the recycling of washing water ozone is superior for improving water clarity. This could be best implemented to decompose and coagulate organic matter prior to filtration. If additional sanitising is required this could be followed by ultraviolet treatment of the clarified water.

4.5 Conclusion

In conclusion, considerable gains may be achieved with respect to product quality and shelf-life. Providing, the washing is continuously monitored and controlled to ensure effective sanitation, and that physical disorders are limited by appropriate equipment selection and washing agents.

Evaluating the performance of the washing of minimally processed vegetables may be best achieved by an industry survey. Which could evaluate the effectiveness of the washing sanitizers and washing equipment. Parameters to be monitor for washing performance must include physiological disorders, microbial spoilage, product shelf-life wash-water quality and the limitation of water usage by recycling. This will require the development of measuring protocols for microbiology, wash-water quality and sanitizers.

5. References:

3M™ Microbiology Products. 1995. *3M Micro Messenger*, Vol. III, 1-4.

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

Anon 1983. Sulphite reducing substitute preserves colour in vegetables. *Food Engineering* **55:** (7) 138.

Anonymous Author. 1995_a. Innovative washing and drying. *Fresh Cut*. November/December, 24, 35.

Anonymous Author 1995_b. High-Tech wash system boosts shelf life. *Fesh Cut*, July/August, 30-31.

Avena-Bustillos, R. J., Krochta J. M., and Saltveit, M. E. 1994 Optimisation of caseinate-based edible coatings on red delicious apples and celery sticks.

Avena-Bustillos, R. J., Cisneros-Zevallos, L. A., Krochta J. M., and Saltveit, M. E., Jr. 1993. Optimisation of edible coatings on minimally processed carrot to reduce white blush using surface response methodology. ASAE, **36:** (3) 801-805.

Baldwin, E. A., Nisperos-Carriedo, M. O. and Baker R. A. 1995. Edible coatings for lightly processed fruits and vegetables. *Journal of Horticultural Science* **30:** (1) 35-38.

Beck, R. G. 1992. Compositions and methods for inhibiting browning of processed produce. United States patent (US5126153).

Beuchat, L. R. 1992. Surface disinfection of raw produce. *Dairy, Food and Environmental Sanitation* **12:** (1) 6-9.

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:** (3) 755-758.

Bolin H. R. and Huxsoll C. C. 1991. Control of minimally processed carrot (Daucus carota) surface discoloration caused by abrasive peeling. *J. Food Sci.* **56:** 416-418.

Brackett, R. E. 1987. Antimicrobial effect of chlorine on *Listeria monocytogenes. J. Food Prot.* **50:** (12) 999-1003.

Brackett, R. E. 1989. Changes in thte microflora of packaged fresh broccoli. *J. Food Quality.* **12:** 169-181.

Brackett, R. E. 1992. Shelf stability and safety of fresh produce as influenced by sanitation and disinfection. *J. Food Prot.* **55:** (10) 808-814.

Bruemmer J. H. 1987. Stability prepared carrot sticks in storage. *Proc. Fla. State Hort. Soc.* **100:** 36-38.

Casquilho M. 1993. Spin Dryer Selection. *Fresh Cut.* Nov/Dec, 14-15.

Couture R. M. L. Cantwell, D. Ke and M. E. Jnr. Saltviet. 1993. Physiological attributes related to quality attributes and storage life of minimally processed lettuce. *Hort. Sci.* **28:** (7) 723-725.

Daiee, K.K., and Okishio, T. 1995. Method of maintaining freshness of cut vegetable. Method of maintaining freshness of garnish of Japanese radish. Method of preserving freshness of cut fruit. *Japanese Patent Applications* 6-245693, 6-245694, 6-245695.

Dziezak J. D. 1988. Monsanto's new product extends produce freshness. *Food Technology* **42:** (9) 98.

Entes P.C. 1985 Apparatus for washing slices of a tuberous plant. Patent Number US 4549478.

Food Industry Sourcebook. 1995. Ed. Bary Pestona, Publ. Minnis Business Press P/L.

Food Processors Guide-Membership Directory. 1987. Corporate Author, Publ. Food Processing Machinery and supplies Association.

Franson A. H., Clesceri L. S., and Greenberg A. E. 1991. Standard methods for the examination of water and waste water, 17th Ed. American Public Health Association, Washington, D. C.

Garg, N., J. J. Churey, and D. F. Splittstoesser. 1990. Effect of processing conditions on the microflora of fresh-cut vegetables. *J. Food Prot.* *53:* (8) 701-703.

Guthrie, B.D. and Beelman, R.B. 1989 Control of bacterial degradation of fresh washed mushrooms. *Mushroom Science* XII. Part II. Proceeding of the twelth international congress on the science and cultivation of edible fungi. Braunschweig, Germany, September, 1987.

Hardy D.M., Kraeger S. J., Dufour S. W. and Cady P. 1977. Rapid detection of microbial contamination in frozen vegetables by automated impedence measurements. *Applied and Environmental Microbiology* **34** (1) 14-17.

Hicks J. R. and Hall C. B. 1972. Control of shredded lettuce discoloration. *Proceedings of the Florida State Horticultural Society* **85**: 219-221.

Hobbs, B. C., and R. J. Gilbert. 1978. Food poisoning and food hygiene, 4th ed. Edward Arnold Publ. Ltd., London. p.366.

Howard L. R. and Griffen L. E. 1993. Lignin formation and surface discolouration of minimally processed carrot sticks. *J. Food Sci.* **58:** 1065-1067, 1072.

Howard L. R., Griffen L. E. and Lee Y. 1994. Steam treatment of minimally processed carrot sticks to control surface discolouration. *J. Food. Sci.* **59:** 356-358.

Humphries C. 1995. The worlds most effective litter removal system. *Pollutec Operations - Water Poulltion Control Pty Ltd.*

Hurst W.C. 1995. Disinfection Methods: A comparison of chlorine dioxide, ozone and ultraviolet light alternatives. *International Fresh-cut Produce Association.* Fall, 4-5.

Hurst, W. A., and G. A. Schuler. 1992. Fresh produce processing - an industry perspective.
J. Food Prot. **55:** (10) 824-827.

International Technical Information Institute. 1985. *Industrial chemicals. Saftey manual for handling and disposal with toxicity and hazard data.*International Technical Information Institute, Tokyo, Japan. p.113-115, 393.

Kim, H. J., and Taub, I. A. 1988 Natick Army Research Technical Report No. TR-88/052, pp. 162-174, US Army Research Institute, Natick, MA, USA.

Kimura T., Umemwa K., Ogawa Y., and Ogawa M.1969 Washing fresh vegetables by supersonic oscillation. Removal of Cu^{2+} from vegetables accompanied by copper-containing pesticides and histological examination of such treated vegetables. *J. Japanese Society Food & Nutrition* **22** (9) 655-659. FSTA, 71-12-J1424.

Kravitz, H. 1994 Self Life: Tipping the scales. *Fresh Cut* July/August, 18-20.

Lozano-De-Gonzalez P.G., Barret D.M., Wrolstad R.E. and Durst R.W. 1993. Enzymatic browning inhibited in fresh and dried apple rings by pineapple juice. *J. Food Sci.* **58:** (2) 399-404.

Lund, B. M. Bacterial spoilage, in *Post -Harvest Pathology of Fruits and Vegetables,* Dennis, C., Ed., Academic Press, London, 1983, 219.

Lyengar R., and McEvily A. J. 1992. Anti-browning agents: alternatives to the use of sulfites in foods. *Trends in Food Science & Technology* March, (3), 60-64.

Martinez M.V. and Whitaker J.R. 1995. The biochemistry and control of enzymatic browning. *Trends in Food Sci. Tech.* **6:** 195-200.

Matsunaga, T., Takahashi, H., Otani, T., Akisawa, S. and Ono, A. 1995. Method for sterilising food. *Japanese Patent Aapplication* 6-327448.

Menzer R. E. and Nelson J. O. 1986. Water and soil pollutants. In: *Casserett and Doull's Toxicology. The Basic Science of poisons.* 3rd Edition Eds. Klassen C. D., Amdur M. O. and Doull J.

Nguyen-the C. and Carlin F. 1994. The microbiology of minimally processed fruits and vegetables. *Critical Reviews in Food Science and Nutrition.* **34:** (4) 371-401.

Palumbo S. A. 1986. Is refrigeration enough to restrain foodborne pathogens? *J. Food Protection.* **49:** (12) 1003-1009.

Produce processing equipment buyer's guide. 1994 *Fresh Cut.* July/August pp. 10-13, 17, 26.

Restaino L., Frampton E.W., Hemphill J. B., and Palnikar P. 1995. Efficacy of ozonated water against various food-related microorganisms. *Applied and Environmental Microbiology* **61:** (9) 3471-3475.

Reyes, V. 1996. Preservation of exposed underground plant structures. Patent PCT/AU95/00416. Australian Patent Office.

Robson R. 1995. Harvest group: value adding through partnerships. *Good Fruits and Vegetables* **6:** (6) 12-19.

Schneider R. 1994_a Keeping wash water cleaner. *Fresh Cut* Jan/Feb 16.

Schneider R. 1994_b Closed vs pen Flume: Is there really an argument here? *Fresh Cut* July/Aug 24.

Schwank D. J. 1992. Method of inhibiting discolouration of produce. United States patent US5087467.

Senter, S. D., N. A. Cox, J. S. Bailey, and W. R. Forbus. 1985. Microbiological changes in fresh market tomatoes during baking operations. *J. Food Sci.* **50:** 254-255.

Silley P. 1991. Rapid Automated Bacterial Impedence Technique (RABIT) *SGM Quarterley* **8** (3) 48-52.

Silvers P. E. 199_{4a} Getting fresh-cut cleaner. *Fresh Cut* May/June 3, 20-21.

Silvers P. E. 1994_b Getting fresh-cut cleaner. *Fresh Cut* May/June 18-20.

Sykes, G. 1965. Disinfection and Sterilisation. Pitman Press, Bath. p. 385-400.

Tatsumi Y., Watada A. E. and Wergin W. P. 1991. Scanning electron microscopy of carrot stick surface to determine causes of white translucent appearence. *J. Food Sci.* **60:** 142-144.

Trueman, J. R.. 1971. The Halogens. In: Inhibition and destruction of the microbial cell. Ed. by W. B. Hugo. Academic Press, London. p. 137-165

Warren, B. Z. 1991. Food preservative compositions. United States patent US4988522.

Wolfe R. L., Stewart M. H., Liang S. and McGuire M. J. 1989. Disinfection of model indicator organisms in a drinking water pilot plant by using peroxone. *Applied and Environmental Microbiology* **55:** (9) 2230-2241.

Wong, D. M. S., Camirand W. M. and Pavalath A. E. 1994 Development of edible coatings for minimally processed fruits and vegetables. In: *Edible coatings and films* *to improve food quality.* Eds. Krochta, J. M., Baldwin, E. A. and Nisperos-Carriedo, M. O. p. 65-88.

Milestone #2: Pilot-scale washing trials on minimally processed vegetables using MCV-resin technology

EXECUTIVE SUMMARY

A pilot-scale trial was undertaken to determine the suitability of using the MCV-resin technology for washing of minimally processed vegetables. The pilot-scale trials established several of the operating parameters needed for integrating the MCV-resin technology into fresh-cut processing.

Operationally the MCV-resin is convenient to use, because the iodine is released into the water on-line at constant levels without the expense required for an iodine dispensing system.

A design and cost consideration is that the resin is only effective in releasing iodine above 10°C where as the water for washing the lettuce should be around 2 to 4°C. This required use of a heat exchanger to cool the water for washing and to heat recycled water prior to entering the resin chamber. Using water at about 12°C the MCV-resin released about 4 ppm iodine.

At this level, the total viable population on fresh-cut samples was reduced 90%, this was the same for *Listeria monocytogenes* and *Salmonella* spp, while for *Escherichia coli* there was an 80% reduction.

With the use of a heat exchanger to temporarily raise the water temperature to 40°C the concentration of iodine released by the MCV-resin increased to about 7.0 ppm. This substantially improved product sanitation, obtaining a 98% reduction in the total viable population on fresh-cut samples.

To improve product sanitation without the requirement of temperature control the MCV-resin could be chemically modified to provide the release of iodine at higher concentrations. The MCV-resin technology can provide a useful chlorine-free washing system for fresh-cuts, avoiding the harmful disinfectant by-products associated with chlorine washing.

The MCV-resin technology should be suitable for treatment of final rinsing water (or spray water) prior to dewatering, due to the low levels and rapid breakdown, as evident from residue testing.

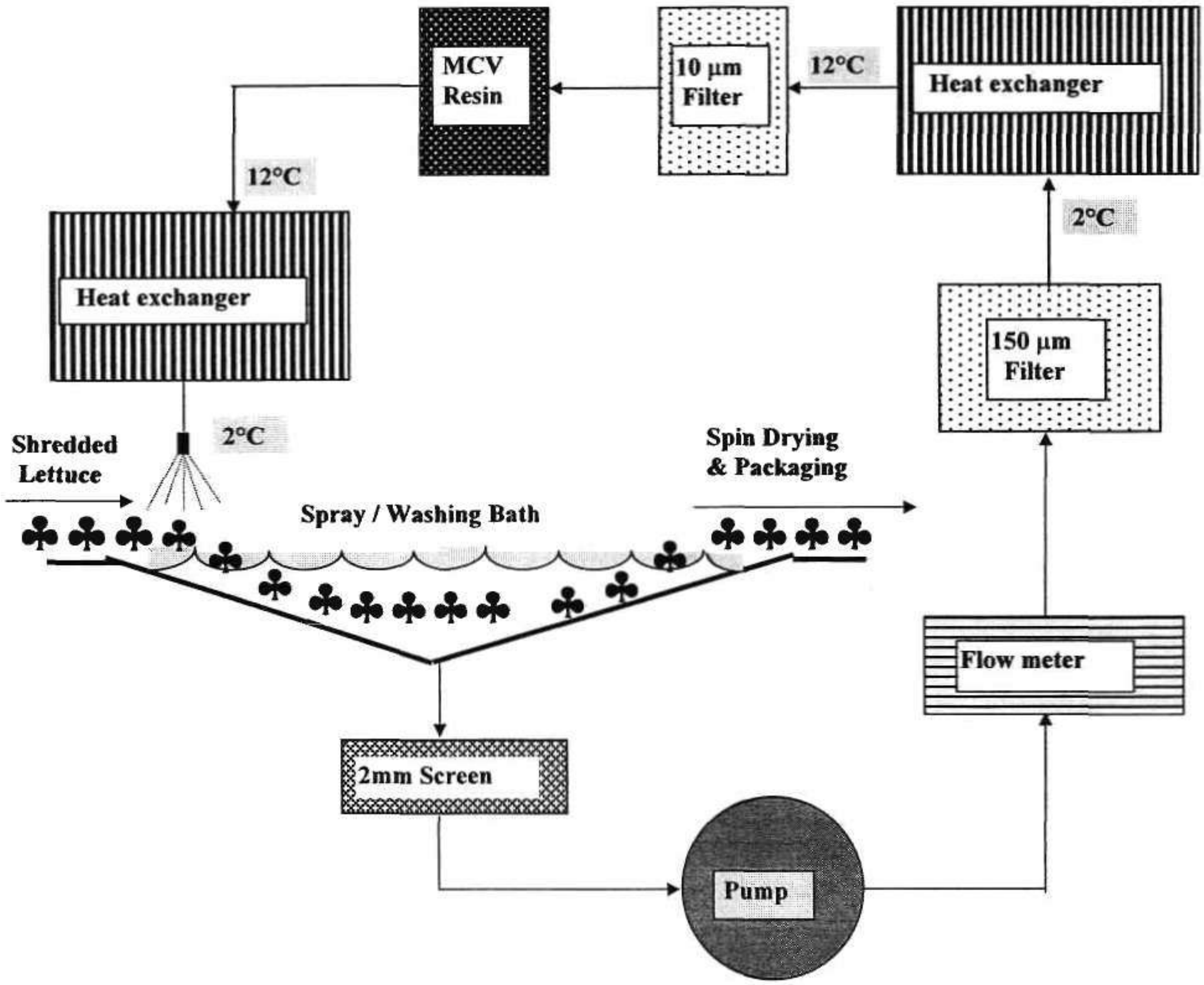
1. Aim

To determine the suitability of MCV-resin technology for the sanitation of fresh-cut vegetables by pilot-scale studies; including establishment of appropriate washing system design, operating conditions, investigating effectiveness against pathogens and residual levels

2. Methodology

2.1 Operational set-up of washing system

Figure 1. MCV-resin washing system



2.2 Summary of operational parameters

Iodine resin: 1.2 litres
Flow rate of recycling water: 700 litres/hour
Product immersion time of 3 minutes (conveyor speed setting of 0.5)
Continual operation of a spray fan jet (200 litres/hour) and 5 air jets.
Volume of washing bath: 60 litres
Total volume of water in recycling system: 120 litres
Filters: 2 mm screen, 150 μm basket filter, 10 μm filter
Pressure: 120 kPa
pH: 7.2-7.6 unadjusted
Product load rate: about 5 kg / 6 minutes
Temperature of water in washing bath 2°C
Temperature of water entering resin chamber 12°C

Note: These are the standard operating parameters except where specifed otherwise in sections3.1, 3.2, 3.3 and 3.4.

2.3 Operational procedure for washing trials

2.3.1 Operational set up of washing system

Set up of the continuous washing / recycling system involved filling with town water, then recycling the water for half an hour to allow for temperature equilibration. During this time the MCV-resin was removed and a charcoal filter placed on-line to remove any residual chlorine present in the town water. Chlorine-free water is recommended because low levels of chlorine effect the rate at which iodine is released from the MCV-resin. After de-chlorination of the washing water using the charcoal filter, it was removed and the MCV-resin replaced. At a water recycling rate of 700 litres per hour (refer Summary of operational procedure) the iodine levels equilibrated within 15 minutes. Testing of the iodine and chlorine levels in the washing water was performed with a rapid colorimetric instruments supplied by Test Equip 2000, using the diethyl-*p*-phenylene diamine (DPD) reaction method.

2.3.2 Product preparation

Shredded lettuce:
Iceberg lettuce was purchased the day before processing and stored overnight at 2°C. The outer leaves and stem were removed, then trimmed of any browned or soiled sections, the heads were halved and the core removed, then shredded into 1 cm wide strips.

Broccoli florets:
Broccoli heads were purchased the day before processing and stored overnight at 2°C. Small florets of about 2-4 cm in diameter were cut from the main stem, suitable for inclusion in vegetable medleys and stir-fries. The main stem was discarded.

Carrot batons:
Carrots were topped and tailed then cut to 6 cm lengths, then abrasively peeled.

2.3.3 Washing, spin drying and packaging

Product was placed on a conveyor which travelled trough the washing bath. Product was pre-sprayed then immersed for 3 minutes. Washed product was spin-dried, then weighed and packaged into permeable polypropylene pouches and sealed using a freshline packaging machine.

2.4 Product Testing

2.4.1 Microbiological analysis

Products were sampled in triplicate for microbiological analysis, 20 grams portions were homogenised (Colworth Stomacher) in 180 mL of 0.1 % bacteriological peptone (Oxoid), then spiral plated on plate count agar (Oxoid) and incubated for 2 days at 30°C.

Pathogen study:
Shredded lettuce was inoculated with either *Salmonella spp, Listeria monocytogens* or *Escherichia spp*, using a dipping solution. Inoculated lettuce was then stored for 5 hours at 10°C, then washed using water treated by MCV-resin technology. Products were sampled in triplicate for microbiological analysis, 20 grams portions were homogenised (Stomacher) in 180 mL of 0.1 % bacteriological peptone (Oxoid), then pour plated using XLD (Oxiod) for *Salmonella spp*, incubated at 37°C for 1 day; Listeria selective agar (Oxiod) for *Listeria monocytogens*, incubated at 30°C for 1 day; and Chromocult agar (Merck) for *Escherichia spp* incubated at 37°C for 1 day.

2.4.2 Visual analysis:

Visual observations were scored as follows 5 = excellent, 4 = very good, 3 = good, 2 = poor and 1 = very poor. A score of 2.5 and above is satisfactory, while below is unsatisfactory.

2.4.3 Residue testing

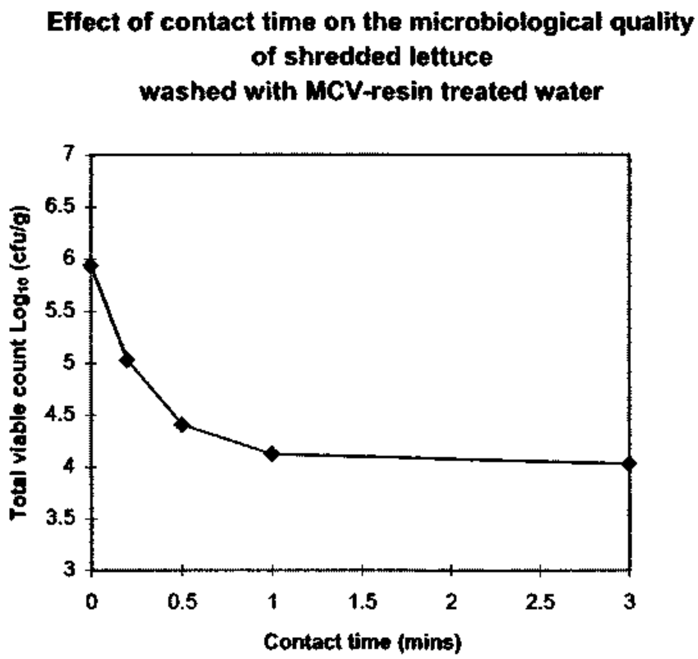
Iodine residue testing was performed within 3 hours from the time of washing. Two 50 gram samples of both unwashed and washed lettuce was rinsed with 100 mL of deionised water and agitated. The level of iodine in washing's was determined using diethyl-p-phenylene. The limit of detection was 0.1 μg/mL, standards were prepared from 0.2 to 2.0 μg/mL.

3. Results

3.1 Effect of contact time (spraying and dipping) on washing performance

The washing contact time was investigated to determine what effect it had on the reduction of microbial populations. The shortest contact time was attained by a single fan-spray which equated to about a 6 second contact period. Longer contact periods were attained by the immersion of the lettuce in the iodinated water for periods of 30, 60 and 120 seconds. The results showed that increasing the immersion period up to 60 seconds improved the microbial kill, however in excess of which provided limited additional effect (see figure2). The same type of scenario would be also likely for the application of additional sprays along a conveyor. Therefore for the MCV-resin system to be most effective the washing system will require a minimum contact time of 1 minute.

Figure 2



3.2 Effect of water temperature on washing performance

Initially the MCV-resin was set up with a single heat exchanger system that cooled the recirculated water. The water temperature was cooled to 10°C, at which the MCV-resin was introduced. At this temperature the iodine level equilibrated at 4.5 ppm. The water temperature was then reduced to 5°C, where the iodine level equilibrated at 3.9 ppm. The air jets were turned on, to provide agitation to the washing water and the system was left operating for 2 hours at 5°C, and iodine levels had dropped to an equilibration level of 3.0 ppm.

In the next trial shredded lettuce was washed in the range of 5.8 to 6.3°C in batches over a period of 3 hours of operation. The iodine level was continually dropping during the operation to 1.5 ppm when the trial was ceased.

It was evident from the first 2 trials that the release of iodine from the resin at 6°C was not sufficient to maintain required levels (ie about 4ppm), however at 10°C was satisfactory. Fresh-cut processors use chilled water in their washing operations at temperatures typically of 1 to 5 °C, which is important to reduce product respiration and microbial growth.

To address this problem a spiral heat exchanger was incorporated into the system, which allowed for the accurate temperature control of water entering the resin, with cooling before entering the washing bath and reheating prior to entering the resin again. For the initial trial with the spiral heat exchanger the water temperature entering the resin was set at 12°C. The water temperature in the washing bath was cooled to 4°C (I_2=5.3ppm), then 2°C (I_2=4.8ppm) where shredded lettuce was washed. Then the temperature was reduced to 0°C at which the water began to freeze (I_2 =3.2ppm). Upon the detection of ice formation the washing water temperature was increase to 1.5 °C and left to run for 3 hours where iodine levels equilibrated at 3.7 ppm. The spiral heat exchanger was therefore successful in maintaining the level of iodine, while using chilled water for washing the produce.

The sanitising performance of iodinated water at 2.5°C was comparable to that at 15°C (Table 1). This is important to fresh-cut processing as it allows for the maintenance of the cold chain and reduces product respiration rate and microbial growth.

Table 1.Comparison of washing lettuce at 15°C and 2.5°C

Samples	Unwashed lettuce (cfu/g)*	15°C Washed lettuce (cfu/g)	2.5°C Washed lettuce (cfu/g)
1	1.82×10^5	5.12×10^4	3.28×10^4
2	3.14×10^5	1.07×10^5	2.46×10^4
3	2.20×10^5	4.07×10^4	2.36×10^4
Average	2.39×10^5	6.63×10^4	2.88×10^4

*colony forming units per gram (cfu/g)

Raising the temperature of the water entering the MCV-resin from 15°C to 41°C was found to increase the rate of iodine release from 4.1 ppm to 7.3 ppm. This resulted in improved product sanitation, increasing the reduction of total viable population from 85% to 98% (Table 2).

Table 2 Effect of iodine concentration on sanitation of shredded lettuce

Shredded lettuce	Unwashed lettuce (cfu/g)	Lettuce washed in 4.1 ppm iodine (cfu/g)	Lettuce washed in 7.3 ppm iodine (cfu/g)
Sample 1	2.85×10^5	1.65×10^4	7.32×10^3
Sample 2	3.50×10^5	5.69×10^4	6.30×10^3
Sample 3	1.71×10^5	4.39×10^4	5.69×10^3
average	**2.69×10^5**	**3.91×10^4**	**6.44×10^3**

*colony forming units per gram (cfu/g)

3.3 Effect of flow rate on washing performance

The washing trials were conducted using a recycling washing system. The flow rate for the recycling water is critical in this system because it has an effect on the concentration of iodine released. The MCV-resin system is designed to release a constant amount of iodine (about 4ppm) into the water stream up to a maximum flow rate where the water residence time in the canister is not sufficient for enough iodine to be released. Flow rates of 250, 500 and 700 litres per hour resulted in iodine levels of approximately 4.0 ppm. However when the flow rate reached 900 and 1200 litres per hour the iodine levels declined to 2.9 and 2.4 ppm respectively. This indicated that a maximum flow rate of about 700 litres per hour is achievable using the system in its current configuration. The maximum flow rate is dependent on the volume of resin, therefore to obtain a higher flow rate a larger volume of resin could be used, or additional MCV-resin canisters situated in parallel.

3.4 Effect of pH on washing performance

When the iodine was released into the water stream the pH declined from 9.0 (town water) to 7.4. Further acidification of the water using citric acid to 4.0, did not result in any sustantial reduction in total viable count (Table 3).

Table 3. Comparison of microbial quality of shredded lettuce washed at pH 7.4 and 4.0

Shredded lettuce	pH 7.4	pH 4.0
Sample 1	1.38×10^4	1.91×10^4
Sample 2	1.57×10^4	1.57×10^4
Sample 3	2.24×10^4	1.61×10^4
Average	**1.73×10^4**	**1.70×10^4**

*colony forming units per gram (cfu/g)
**adjusted to 4.0 using the addition of 1% citric acid

3.5 Washing of shredded lettuce, carrot batons and broccoli florets.

The total viable count for shredded lettuce, broccoli florets and carrot batons was reduced by 88%, 88% and 79% respectively (Tables 4, 5,and 6).

Table 4. Total viable count of washed shredded lettuce

Samples	Unwashed lettuce (cfu/g)*	Washed lettuce (cfu/g)
1	8.74×10^3	1.42×10^3
2	1.18×10^4	1.42×10^3
3	1.79×10^4	1.63×10^3
Average	**1.28×10^4**	**1.49×10^3**

*colony forming units per gram (cfu/g)

Table 5. Total viable count of washed broccoli florets

Samples	Unwashed broccoli (cfu/g)*	Washed broccoli (cfu/g)
1	3.28 x 10^6	2.20 x 10^5
2	3.74 x 10^6	4.23 x 10^5
3	2.61 x 10^6	5.33 x 10^5
Average	**3.21 x 10^6**	**3.92 x 10^5**

*colony forming units per gram (cfu/g)

Table 6. Total viable count of washed carrot batons

Samples	Unwashed carrots (cfu/g)*	Washed carrots (cfu/g)
1	2.12 x 10^5	9.73 x 10^4
2	3.65 x 10^5	4.96 x 10^4
3	4.67 x 10^5	6.40 x 10^4
Average	**3.40 x 10^5**	**7.03 x 10^4**

*colony forming units per gram (cfu/g)

3.6 Effect of washing on pathogens, *Listeria monocytogenes, Salmonella spp.*, and *Escherichia coli.*

MCV-resin treated water reduced populations of *Listeria monocytogenes, Salmonella spp., Escherichia coli* on artificially contaminated shredded lettuce. The levels of *Listeria monocytogenes, Salmonella spp., Escherichia coli* were reduced 89%, 93% and 80% respectively.

***Escherichia coli* challenge trial:**

Shredded lettuce was inoculated with *Escherichia coli* at about 7.21x 10^3 cfu/g. The level of inoculation is considered high and may occur on rare occasions with fresh-cut product. The iodine treated water was generated using MCV-Resin technology and used within 15 minutes after collection. Following washing of the inoculated shredded lettuce the *Escherichia coli* population was reduced by 80% (Table 7). Following 4 days of storage at 10°C the *Escherichia coli* populations increased to 6.87 x 10^5 cfu/g. Washing the lettuce in the iodine treated water reduced the population of *Escherichia coli*, but did not retard the subsequent growth during storage (Table 7).

Table 7. Summary of *E.coli* challenge trial results

Shredded Lettuce	Control (cfu/g)*	Before washing (cfu/g)	After washing (cfu/g)	After 4 days at 10°C (cfu/g)
Sample 1	< 1.0 x10^1	8.53 x 10^3	1.40 x 10^3	6.04 x 10^5
Sample 2	< 1.0 x10^1	5.88 x 10^3	1.44 x 10^3	7.69 x 10^5
Average	**< 1.0 x10^1**	**7.21x 10^3**	**1.42 x 10^3**	**6.87 x 10^5**

* colony forming units per gram (cfu/g)

The storage temperature of 10°C used in this study is considered an "abuse" condition because fresh-cut products are generally stored at 4-6°C. If appropriate temperature control is maintained (<6°C) then most pathogens will not grow, except for psychrotrophic pathogens such as *Listeria monocytogenes*.

***Salmonella spp.* challenge trial:**

Shredded lettuce was inoculated with *Salmonella spp.* at about 9.4 x 10^2 cfu/g. The level of inoculation is considered high and may occur on rare occasions with fresh-cut products. The iodine treated water was generated using MCV-Resin technology and used within 15 minutes after collection. Following washing of the inoculated shredded lettuce the *Salmonella spp.* population was reduced by about 93 %. Following 4 days of storage at 10°C, the *Salmonella spp.* increased to 2.21 x 10^3 cfu/g. Washing the lettuce in the iodine treated water reduced the population of *Salmonella spp*, but did not retard subsequent growth during storage (Table 8).

Table 8. Summary of *Salmonella* challenge trial results

Shredded Lettuce	Control (cfu/g)*	Before washing (cfu/g)	After washing (cfu/g)	After 4 days at 10°C (cfu/g)
Sample 1	< 1.0 x 10^1	1.06 x 10^3	6.0 x 10^1	2.51 x 10^3
Sample 2	< 1.0 x 10^1	8.2 x 10^2	7.0 x 10^1	1.90 x 10^3
Average	**< 1.0 x 10^1**	**9.4 x 10^2**	**6.5 x 10^1**	**2.21 x 10^3**

* colony forming units per gram (cfu/g)

The storage temperature of 10 °C used in this study is considered an "abuse" condition because fresh-cut products are generally stored at 4-6°C. If appropriate temperature control is maintained (< 6°C) then most pathogens will not grow, except for psychrotrophic pathogens such as *Listeria monocytogenes*.

***Listeria monocytogenes* challenge trial:**

Shredded lettuce was inoculated with *Listeria monocytogenes* at about 1.96 x 10^6 cfu/g. The level of inoculation is considered very high and not likely to occur on fresh-cut products. The iodine treated water was generated using MCV-Resin technology and used within 15 minutes after collection. Following washing of the inoculated shredded lettuce the *Listeria monocytogenes* population was reduced by about 89 % (refer table 9).

Table 9. Summary of *Listeria monocytogenes* challenge trial results

Shredded Lettuce	Control (cfu/g)*	Before washing (cfu/g)	After washing (cfu/g)
Sample 1	< 1.0 x 10^1	1.03 x 10^6	2.61 x 10^5
Sample 2	< 1.0 x 10^1	3.29 x 10^6	2.04 x 10^5
Sample 3	< 1.0 x 10^1	1.58 x 10^6	1.58 x 10^5
Average	**< 1.0 x 10^1**	**1.97 x 10^6**	**2.22 x 10^5**

* colony forming units per gram (cfu/g)

3.7 Residue testing for iodine levels on washed shredded lettuce

Residual iodine levels on washed shredded lettuce:

Iodine was not detected in the water washing from the lettuce leaf surfaces at a detection limit of 0.1 μg/mL (Table 10).

Table 10. Detection of iodine on shredded lettuce

Shredded lettuce samples	Iodine*
Unwashed	Not Detected
Unwashed	Not Detected
Washed	Not Detected
Washed	Not Detected

*limit of detection was 0.1 μg/mL

Recovery of iodine from spiked washing water:

A recovery using 10 μg/mL iodine wash solution was carried out. The resultant solution after following the same procedure for the test samples was significantly lower in iodine than the wash solution (at least 50% less).

The recovery trial demonstrated a rapid depletion of iodine levels, furthermore the low levels of iodine used in the washing process, resulted in levels of iodine on the product surface that are very low (< 0.2 μg/gram of surface washed lettuce). The results of residual iodine on shredded lettuce have shown that the residue levels are below the detection limit of 0.1 μg/mL, using a surface wash method to retrieve the iodine. The rapid depletion of iodine levels post-washing and the resulting low level residuals on the product, could enhance the suitability of this product for application to the fresh-cut industry. Residue testing supported by appropriate toxicological data will be useful in attaining registration of the washing system.

3.8 Comparison of batch washing and a continuous water recycling system

A comparison of batch and continuous washing trials was conducted to determine which was the most appropriate system for use with the MCV-resin washing technology.

The amount of product that could be washed by batch processing was limited due to the rapid depletion of iodine from 5.0 ppm to 1.2 ppm (Figure 3) and resulted in reduced microbial kill (Figure 4). This coincided with total viable count in the water exceeding 10 cfu / mL, and ATP readings exceeding 50,000 RLU. The reduction in total microbial population on the shredded lettuce was 88%, compared with 96% when using freshly treated washing water. In a commercial operation this would necessitate replacement with freshly iodinated washing water.

Using the continuous washing system the iodine level was reduced briefly to 2.0 ppm, but rapidly stabilised at levels of about 3.0 ppm (Figure 3). Even though the total viable count in the water reached 46 cfu / mL, and ATP readings of 82,000 RLU, the reduction in total microbial population on the shredded lettuce of 96% was comparable to that achieved when batch washing with freshly iodinated wate r(Figure 4). At the completion of the washing trial the recycled water prior to contact with the product had an iodine level of 3.5 ppm compared with 5.0 ppm prior to commencement of washing. The reduction in the iodine levels is likely to result from a build up of organic matter in recycled water depleting available iodine. The recycled water was filtered in three stages down a 10 μm cut-off, however this still did not remove cellular fluid released by damaged cells. It can be concluded that the MCV-resin system is more effective for washing when operated using a water recycling system.

Figure 3

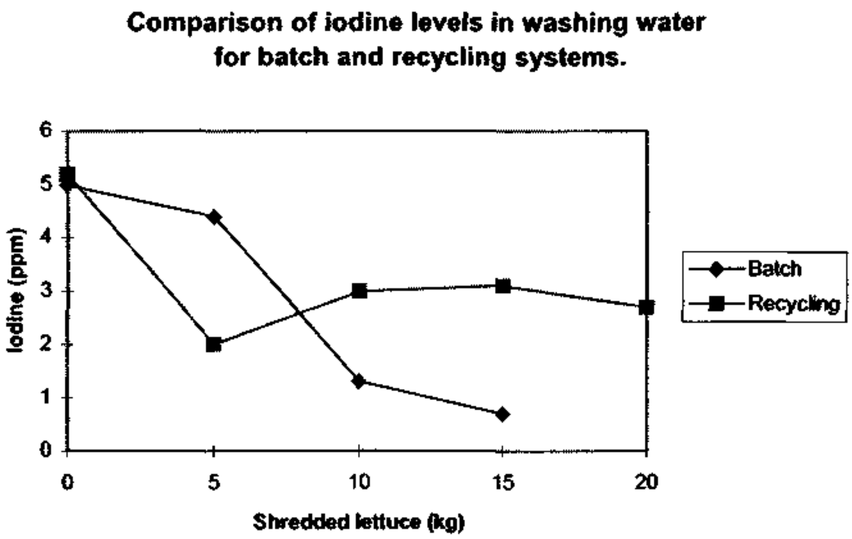
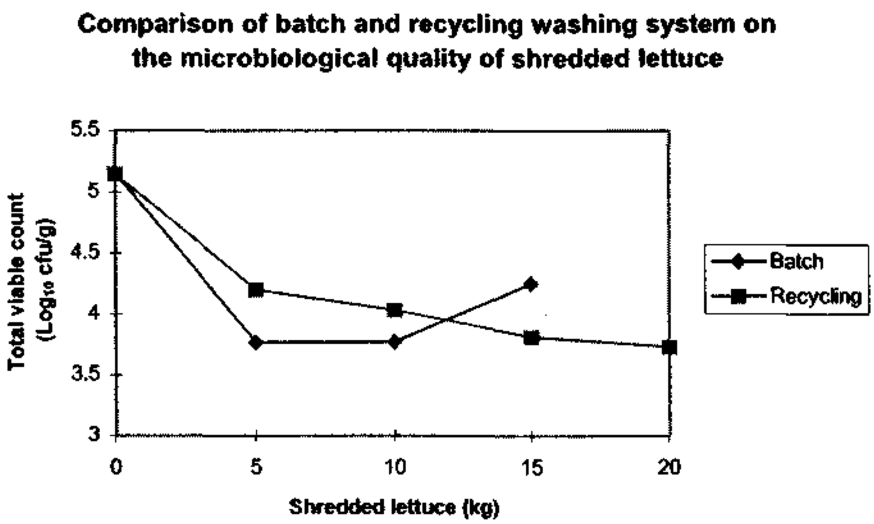


Figure 4



4. Discussion

The pilot-scale trials established operating parameters valuable to industry for integrating the MCV-resin technology into the fresh-cut industry. Operationally the MCV-resin is convenient to use, because the iodine is released into the water on-line at constant levels without the expense required for an online monitoring and dispensing system. However, intermittent determination of iodine levels is recommended as this is one of the critical control points for a HACCP system. Rapid iodine monitoring systems suitable for use by processing staff are available.

Chlorine free water is required because low levels of chlorine effect the rate at which iodine is released from the MCV-resin. Therefore the MCV-resin system could not be used in conjunction with a chlorine washing step, where there would be a carry over of chlorine contaminating water that contacts the MCV-resin. However, if the iodinated water is sprayed onto the product or a batch washing system is used, then this problem is avoided.

The level of iodine released is limited to 4 to5 ppm, which is a limitation upon product sanitation. Therefore the MCV-resin technology will not provide sanitation comparable to that achieved with high levels of chlorine currently used by the fresh-cut industry of about 100ppm.

5. Key Findings

*Minimum contact time of 1 minute; therefore not as suitable for fast flumes for which the contact period is less the 30 seconds (refer 3.1).

*Temperature of water passing the MCV-resin needs to be at least 10 °C, to ensure iodine levels are maintained in a recycling washing system (refer 3.2). Preferably, water is chilled prior to entering washing bath (2-4°C) and heated to about 12°C prior to passing through the resin.

*For a system using 1.2 litres of MCV-resin the maximum recommended flow rate was about 700 litres/hour to maintain 4 ppm iodine in the washing tank (refer 3.3). If a higher flow rate is required then a larger volume of resin could be used or the set-up of additional canisters in parallel.

*Acidification of the washing water did not improve sanitation effectiveness (refer 3.4).

*Applicable for the final rinse or spray stage prior to de-watering due to the low levels and rapid breakdown, as evident from residue testing (refer 3.6).

*Generally the total viable population on fresh-cut samples was reduced 90% (refer 3.5), this was the same for Listeria monocytogenes and Salmonella spp, while for Escherichia coli there was an 80% reduction (refer 3.6).

*The level of iodine released by the MCV-resin was increased to 7.3 ppm when the water temperature was raised to 41°C. This improved the reduction in total viable population on fresh-cut samples to 98%.

Milestone #3: Pilot-scale washing trials on minimally processed vegetables using Tsunami™

EXECUTIVE SUMMARY

Pilot-scale trials were undertaken to determine the suitability of using Tsunami™ for the washing of minimally processed vegetables. Tsunami™ has recently been marketed in the US for the treatment of flume water. Presently this product has not been marketed in Australia, because it still has to be registered with the NRA and approved for use as a washing agent by ANZFA. The product was supplied by Ecolab with US performance data based upon the microbiological quality of washing water. Ecolab was keen to see an Australian evaluation undertaken for the fresh-cut industry and supplied product for the CRC washing trial. The CRC sought to perform an extensive evaluation to determine the effectivness of Tsunami™ in improving microbiological quality of the product, including challenge trials with human pathogens onto artificially inoculated shredded lettuce.

Firstly a dose-response study was undertaken, encompassing levels well in excess and below that recommended by Ecolab. This demonstrated that Tsunami™ was effective in sanitising shredded lettuce even at 5 ppm. However, recommended usage levels of at least 10 ppm are recommended to maintain microbiological quality of water and POAA availability as the organic load increases in the washing bath. The batch washing trial demonstrated that the POAA level decreased at a similar rate to that of free available chlorine, throughout an extended production trial. Contact time studies demonstrated that the reduction in viable count could largely be achieved by spraying or an immersion of only 30 seconds. An extended immersion time did not provide additional microbial reduction. This indicates that Tsunami™ could be effective in fast moving flumes or applied as sprays. Generally the contact time used in industry is longer (1-2 minutes) to ensure proper mixing and exposure to all surfaces. The pilot-scale washing trials generally achieved a reduction in the total viable count on shredded lettuce of 90 to 99%. Chilled water of 2-3°C was used which is favourable as chilling reduces produce respiration rates and microbial growth. Tsunami™ was more effective in reducing the viable numbers of *Listeria monocytogens* on the lettuce surface, followed by *Salmonella spp.*, and least effective against *E.coli.* For shredded lettuce artificially inoculated with *Listeria monocytogenes, Salmonella spp.,* and *E.coli* the viable numbers were reduced by 97.2%, 92.4% and 87.8% respectively by washing with 10 ppm POAA, and by 99.6%, 95.6% and 93.4% respectively using 50 ppm POAA.

The Tsunami™ was very easy to use, it involved dispensing a small amount of concentrate into the washing water, in the same manner as adding chlorine for a batch washing operation. Peracetic acid is the main active ingredient which was conveniently monitored using analytical test strips (Merck). This technique is very practical for rapid testing and cost effective. Titration to determine POAA levels can be performed, but require specialist training and considerable time before results are provided. For a continuous washing system online monitoring would be much more practical. Following discussions with Ecolab, the use of an oxidative redox potential (ORP) probe to monitor peracetic acid (POAA) level and control dosage of the

washing tank was favoured based upon trials in the US. The intention is to evaluate the ORP control system in our pilot-scale processing facility as this would be critical for the adoption of Tsunami™ by anyone of the larger fresh-cut facilities in Australia, which already operate continuous washing systems.

1. Aim

To determine the suitability of Tsunami™ for the sanitation of fresh-cut vegetables by pilot-scale studies; including establishment of appropriate washing system design, operating conditions, investigating effectiveness against pathogens.

2. Methodology

2.1 Operational procedure for washing trials

2.1.1 Operational set up of washing system

Set up of the continuous washing / recycling system involved filling the washing bath and overfill tank with town water. The water was then cooled to 2-3 °C by recycling through a plate heat exchanger. Tsunami™ was added to the washing bath and overfill tank in an even distribution while the water was recycling to achieve thorough mixing. Then the peracetic acid (POAA) concentration was determined using test strips (Merck).

2.1.2 Summary of operational parameters

Flow rate of recycling water: 700 litres/hour
Product immersion time of 3 minutes (conveyor speed setting of 0.5)
Continual operation of a spray fan jet (200 litres/hour) and 5 air jets.
Volume of washing bath: 60 litres
Volume of overfill tank: 40 litres
Total volume of water in recycling system: 120 litres
Filters: 2 mm screen, 150 µm basket filter, 10 µm filter
Pressure: 120 kPa
pH: 6.0-7.0
Product load rate: about 5 kg / 6 minutes
Temperature of water in washing bath 2-3°C
Tsunami™ 100 (supplied by Ecolab)

Note: These are the standard operating parameters except where otherwise specified.

2.2 Product preparation

Shredded lettuce:
Iceberg lettuce was purchased the day before processing and stored overnight at 2°C. The outer leaves and stem were removed, then trimmed of any browned or soiled sections, the heads were halved and the core removed, then shredded into 1 cm wide strips.

Broccoli florets:
Broccoli heads were purchased the day before processing and stored overnight at 2°C. Small florets of about 2-4 cm in diameter were cut from the main stem, suitable for inclusion in vegetable medleys and stir-fries. The main stem was discarded.

Carrot batons:
Carrots were topped and tailed then cut to 6 cm lengths, then abrasively peeled and rinsed with tap water to remove peeled particles.

2.3 Washing, spin drying and packaging

Product was placed on a conveyor which travelled trough the washing bath. Product was pre-sprayed then immersed for 3 minutes. Washed product was spin-dried, then weighed and packaged into permeable polypropylene pouches and sealed using a freshline packaging machine.

2.4 Product Testing

2.4.1 Microbiological analysis

Products were sampled in triplicate for microbiological analysis, 20 grams portions were homogenised (Colworth Stomacher) in 180 mL of 0.1 % bacteriological peptone (Oxoid), then spiral plated on plate count agar (Oxoid) and incubated for 2 days at 30°C.

Pathogen study:
Shredded lettuce was inoculated with either *Salmonella spp, Listeria monocytogens* or *Escherichia spp*, using a dipping solution. Inoculated lettuce was then stored for 5 hours at 10°C, then washed using Tsunami™ treated water. Products were sampled in triplicate for microbiological analysis, 20 grams portions were homogenised (Stomacher) in 180 mL of 0.1 % bacteriological peptone (Oxoid), then pour plated using XLD (Oxiod) for *Salmonella spp*, incubated at 37°C for 1 day; Listeria selective agar (Oxiod) for *Listeria monocytogens*, incubated at 30°C for 1 day; and Chromocult agar (Merck) for *Escherichia spp* incubated at 37°C for 1 day.

3. Results

3.1 Dose response study

Shredded lettuce was washed with different concentrations of Tsunami™ to determine the effect of dose on the microbial quality of the product. An improvement the microbial quality of the lettuce was achieved by increasing the peracetic acid (POAA) concentration from 25 ppm to 125 ppm.

The microbial reduction of 96% at 5 ppm exceeded that recorded at 25, 40 and 80 ppm, which is unusual (see table 1). This may have resulted from the natural variation of microbial load between inner and outer leaves on the lettuce. Though this demonstrates that the Tsunami™ product maybe very effective at lower concentrations, providing good quality product is used.

Recommendations of flume water concentrations from Ecolab® is 5-10 ppm or higher, based upon maintaining microbiological water quality (> 1.0 log reduction). In the case of bacteria attached to food surfaces they are harder to kill then free floating cells, thus higher POAA are likely to be necessary. There was a clear benefit in using the highest dose at 120 ppm but it may well be financially uneconomical to use at such high levels. This will be investigated in the cost benefit report to be completed in June 1998.

Table 1. Effect of Tsunami™ washing concentration on the microbial quality of shredded lettuce.

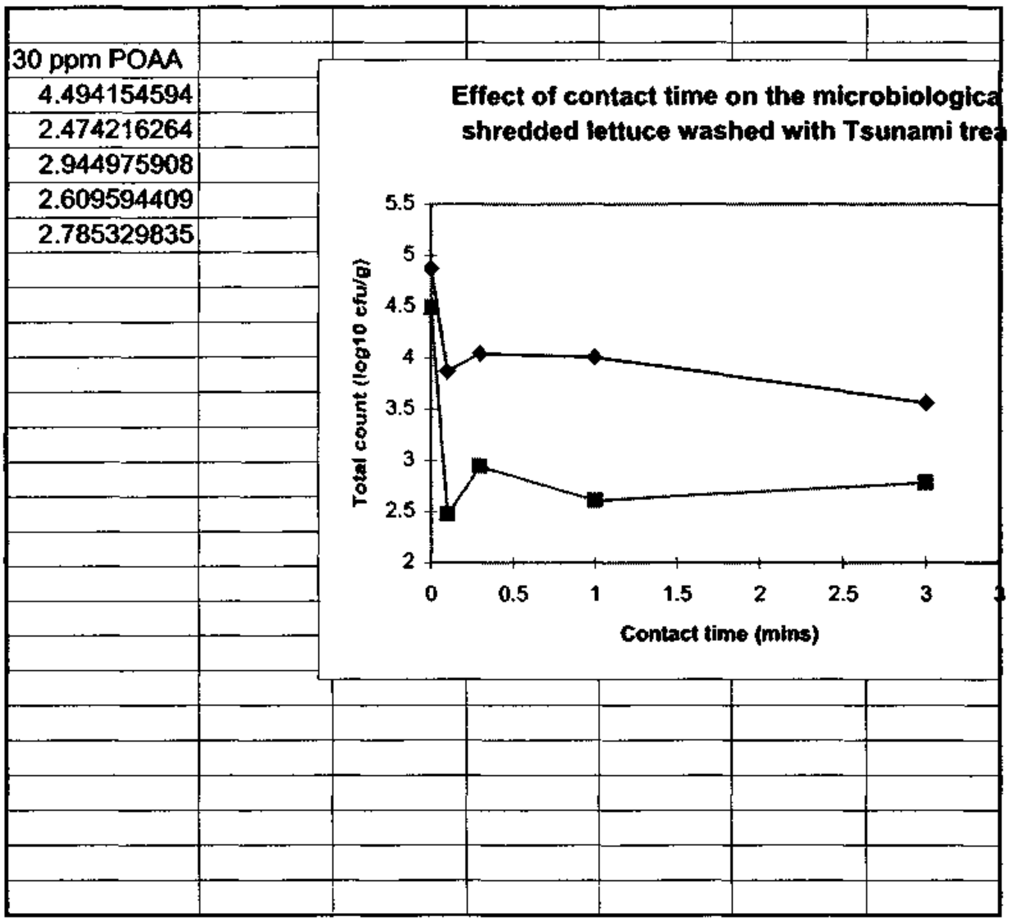
Lettuce Treatments	Total count (cfu/g)	Average (cfu/g)	% Reduction
Unwashed lettuce #1 " " #2	2.27 x10^5 2.65 x10^5	2.46 x10^5	—
Water wash #1 " " #2	1.40 x10^5 1.23 x10^5	1.32 x10^5	46 %
Tsunami POAA 5ppm #1 " " " #2	1.18 x10^4 1.02 x10^4	1.10 x10^4	95.5 %
Tsunami POAA 25ppm #1 " " " #2	6.27 x10^4 1.12 x10^4	3.70 x10^4	85 %
Tsunami POAA 40ppm #1 " " " #2	3.07 x10^4 2.72 x10^4	2.90 x10^4	88.2 %
Tsunami POAA 80ppm #1 " " " #2	1.65 x10^4 2.03 x10^4	1.84 x10^4	92.5 %
Tsunami POAA 120ppm #1 " " " #2	1.21 x10^3 1.42 x10^3	1.32 x10^3	99.5 %
Free Chlorine 50ppm #1 " " " #2	1.16 x10^4 1.12 x10^4	1.14 x10^4	95.1 %

3.2 Effect of contact time (spraying and dipping) on washing performance

The washing contact time was investigated to determine what effect it had on the reduction of microbial populations. The shortest contact time was attained by a single fan-spray which equated to about a 6 second contact period. Longer contact periods were attained by the immersion of the lettuce in the Tsunami™ treated water (POAA, 30 ppm) for periods of 30, 60 and 120 seconds.

Tsunami™ treated water was found to be as effective in reducing the microbial load with spraying or a short dipping as compared with prolonged immersion (see...) This trial was repeated at a POAA concentration of 5 ppm for which the microbial reduction from spraying (90%) exceeded the 30 and 60 second wash possibly due to the continued application of freshly treated water to the lettuce surface. Where as for the immersion process there would have been some depletion of available peracetic acid as the batch of lettuce proceeded through the washing bath, this would reduce microcidal effectiveness. With increasing immersion times the microbial reduction was increased to 95% at 3 minutes which exceeded that achieved by spraying. The trial at a lower POAA limit of 5ppm, demonstrated a benefit in the extension of the immersion time to 180 seconds to maximise microbial reduction.

Figure 1



3.3 Washing of shredded lettuce, carrot batons and broccoli florets.

The total viable count for shredded lettuce, broccoli florets and carrot batons washed with Tsunami™ at POAA concentration of 20 ppm, was reduced by 95.9 %, 90.5 % and 78.1% respectively (Tables 2, 3,and 4). The microbial reduction for the carrot batons was lower because the batons were prewashed in water to remove any residual peel.

Table 2. Total viable count of washed shredded lettuce

Samples	Unwashed lettuce (cfu/g)*	Washed lettuce (cfu/g)
1	1.01×10^5	8.54×10^3
2	1.63×10^5	8.33×10^3
3	2.61×10^5	4.88×10^3
Average	**1.75×10^5**	**7.25×10^3**

*colony forming units per gram (cfu/g)

Table 3. Total viable count of washed broccoli florets

Samples	Unwashed broccoli (cfu/g)*	Washed broccoli (cfu/g)
1	3.09×10^6	1.27×10^5
2	2.83×10^6	4.23×10^5
3	2.28×10^6	2.27×10^5
Average	**2.73×10^6**	**2.59×10^5**

*colony forming units per gram (cfu/g)

Table 4. Total viable count of washed carrot batons

Samples	Unwashed carrots (cfu/g)*	Washed carrots (cfu/g)
1	4.55×10^4	1.36×10^4
2	6.80×10^4	1.97×10^4
3	9.85×10^4	1.32×10^4
Average	**7.07×10^4**	**1.55×10^4**

*colony forming units per gram (cfu/g)

3.4 Effect of washing on pathogens, *Listeria monocytogenes, Salmonella spp.,* and *Escherichia coli.*

Tsunami™ treated water reduced populations of *Listeria monocytogenes, Salmonella spp., Escherichia coli* on artificially contaminated shredded lettuce. The levels of *Listeria monocytogenes, Salmonella spp., Escherichia coli* were reduced by 97.2 %, 92.4 % and 87.8 % respectively using 10 ppm POAA, and by 99.6%, 95.6% and 93.4% using 50 ppm POAA.

***Escherichia coli* challenge trial:**

Shredded lettuce was inoculated with *Escherichia coli* at about 7.34×10^6 cfu/g. The level of inoculation is considered high with fresh-cut product. The shredded lettuce was washed with Tsunami™ treated water at POAA concentrations of 10 ppm and 50 ppm. Following washing of the inoculated shredded lettuce the *Escherichia coli* population was reduced by 87.8 % and 93.4 % using 10 and 50 ppm POAA respectively (Table 5).

Table 5. Summary of *E.coli* challenge trial results

Lettuce Treatment	E.coli (cfu/g)*	Average (cfu/g)	% Reduction
Unwashed (blank) #1	$< 1 \times 10^1$		
" " #2	$< 1 \times 10^1$	$< 1 \times 10^1$	—
" " #3	$< 1 \times 10^1$		
E.coli innoculated (control) #1	6.05×10^6		
" " " #2	8.23×10^6	7.34×10^6	—
" " " #3	7.73×10^6		
Tsunami™ POAA 10ppm #1	1.19×10^6		
" " " #2	8.80×10^5	8.98×10^5	87.8 %
" " " #3	6.24×10^5		
Tsunami™ POAA 50ppm #1	3.70×10^5		
" " " #2	4.56×10^5	4.81×10^5	93.4 %
" " " #3	6.18×10^5		

* colony forming units per gram (cfu/g)

***Salmonella spp.* challenge trial:**

Shredded lettuce was inoculated with *Salmonella spp* at about 1.44×10^5 cfu/g. The level of inoculation is considered high with fresh-cut product. The shredded lettuce was washed with Tsunami™ treated water at POAA concentrations of 10 ppm and 50 ppm. Following washing of the inoculated shredded lettuce the *Salmonella spp* population was reduced by 92.4 % and 95.6 % using 10 and 50 ppm POAA respectively (Table 6).

Table 6. Summary of *Salmonella* challenge trial results

Lettuce Treatment	Salmonella (cfu/g)*	Average (cfu/g)	% Reduction
Unwashed (blank) #1	$< 1 \times 10^1$		
" " #2	$< 1 \times 10^1$	$< 1 \times 10^1$	—
" " #3	$< 1 \times 10^1$		
Salmonella innoculated (control) #1	1.65×10^5		
" " " " #2	1.24×10^5	1.44×10^5	—
" " " " #3	1.42×10^5		
Tsunami™ POAA 10ppm #1	9.20×10^3		
" " " #2	1.60×10^4	1.10×10^4	92.4 %
" " " #3	7.90×10^3		
Tsunami™ POAA 50ppm #1	5.60×10^3		
" " " #2	7.52×10^3	6.29×10^3	95.6 %
" " " #3	5.76×10^3		

* colony forming units per gram (cfu/g)

***Listeria monocytogenes* challenge trial:**

Shredded lettuce was inoculated with *Listeria monocytogenes* at about 1.65 x 10^5 cfu/g. The level of inoculation is considered high with fresh-cut product. The shredded lettuce was washed with Tsunami™ treated water at POAA concentrations of 10 ppm and 50 ppm. Following washing of the inoculated shredded lettuce the *Listeria monocytogenes* population was reduced by 97.2 % and 99.6 % using 10 and 50 ppm POAA respectively (Table 7).

Table 7. Summary of *Listeria monocytogenes* challenge trial results

Lettuce Treatment	L.monocytogenes (cfu/g)*	Average (cfu/g)	% Reduction
Unwashed (blank) #1 " " #2 " " #3	< 1 x 10^1 < 1 x 10^1 < 1 x 10^1	< 1 x 10^1	—
L.monocytogenes innoculated (control) #1 " " " " " #2 " " " " " #3	1.5 x 10^5 1.9 x 10^5 1.6 x 10^5	1.7 x 10^5	—
Tsunami™ POAA 10ppm #1 " " " #2 " " " #3	3.6 x 10^3 4.2 x 10^4 6.0 x 10^3	4.6 x 10^3	97.3 %
Tsunami™ POAA 50ppm #1 " " " #2 " " " #3	6.6 x 10^2 6.7 x 10^2 8.8 x 10^3	7.4 x 10^2	99.6 %

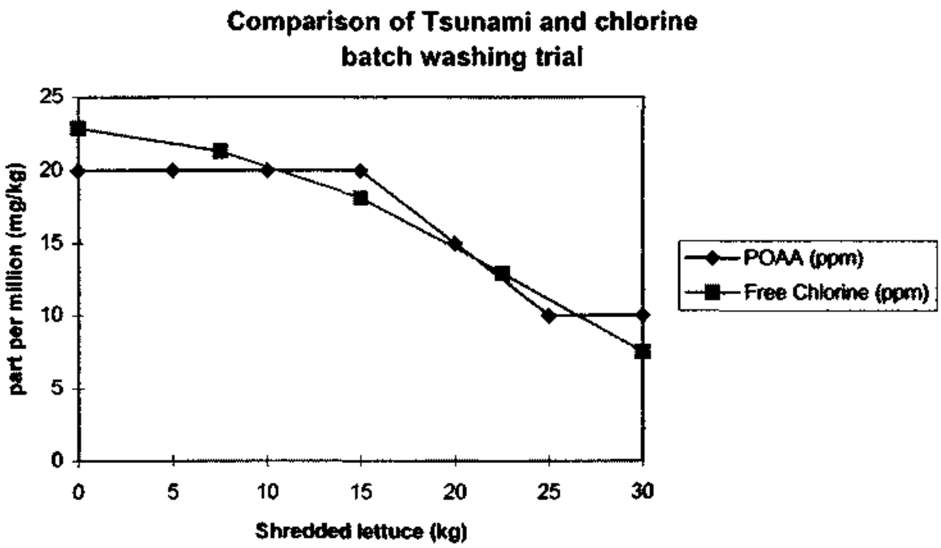
* colony forming units per gram (cfu/g)

3.5 Batch washing trial: a comparison between Tsunami™ and chlorine washing

A batch washing trial was performed with shredded lettuce to determine washing performance with increasing organic load in the washing bath. Washing performance indicators included the measurement of peracetic acid concentration, water quality by ATP-bioluminescence and microbial count, and the microbial quality of the product. These tests were performed and samples collected at 5 kg intervals of washed shredded lettuce for a total of 30 kg.

At commencement of the batch washing trial the POAA concentration was 20 ppm, this level was maintained for the washing of 15 kg, after which a decline of POAA levels was noticeable. Following the washing of 25 kg of shredded lettuce the POAA concentration had halved to 10 ppm (see figure 2). Over this washing period a control water sample still retained a POAA concentration of 20 ppm. The chlorine and POAA were both depleted at a similar rate (see figure 2), in the presence of increasing organic the washing water. The POAA levels were determined using test strips (Merck) with indicator levels of 5, 10, 20, 30 and 50 ppm. Therefore it was not possible to accurately determine the rate of POAA depletion with respect to product load. However, the POAA test strips provide a cost effective convenient indicator method that could be utilised by processors. For a more accurate measurement of the POAA levels an oxidation redox potential (ORP) probe is going to be trialed in later experiments with a continuous washing system to provide online monitoring and dispensing of the POAA levels. The other alternative is to measure the POAA levels by titration.

Figure 2



Throughout the batch washing trial a microbial reduction of > 1 Log was achieved, ranging from 92.1 % to 96.6 %. Even though there was a halving of the POAA concentration to 10 ppm a microbial reduction of 92.1% was still achieved after a total load of 30 kg had been washed (see table 8). This compared favourably with the initial microbial reduction of 95.9% for the first 5 kg batch of washed shredded lettuce. The microbial reduction was similar to that achieved in the chlorine batch washing trial which ranged from 87.2 % to 97.4 % (see table 9)

Figure 8. Microbial quality of shredded lettuce from Tsunami™ batch washing trial

Lettuce Treatments	Total count (cfu/g)	Average (cfu/g)	% Reduction
Unwashed lettuce #1	1.05×10^5		
" " #2	1.63×10^5	1.75×10^5	—
" " #3	2.61×10^5		
5 kg washed lettuce#1*	8.54×10^3		
" " " " #2	8.33×10^3	7.25×10^3	95.9 %
" " " " #3	4.88×10^3		
10 kg washed lettuce#1	3.05×10^3		
" " " " #2	7.72×10^3	6.16×10^3	96.5 %
" " " " #3	7.72×10^3		

20 kg washed lettuce#1 " " " " #2 " " " " #3	7.72×10^3 5.28×10^3 4.67×10^3	5.89×10^3	96.6 %
30 kg washed lettuce #1 " " " " #2 " " " " #3	6.70×10^3 2.36×10^4 1.14×10^4	1.39×10^4	92.1 %

* colony forming units per gram (cfu/g)

Figure 9. Microbial quality of shredded lettuce from chlorine batch washing trial

Lettuce Treatments	Total count (cfu/g)	Average (cfu/g)	% Reduction
Unwashed lettuce #1 " " #2 " " #3	3.98×10^4 1.04×10^4 1.93×10^4	2.32×10^4	—
7.5 kg washed lettuce #1* " " " " #2 " " " " #3	4.06×10^3 1.83×10^3 3.05×10^3	7.25×10^3	87.2 %
15 kg washed lettuce #1 " " " " #2 " " " " #3	6.01×10^2 2.01×10^2 1.02×10^3	6.07×10^2	97.4 %
22.5 kg washed lettuce #1 " " " " #2 " " " " #3	8.13×10^2 4.07×10^2 1.22×10^3	8.13×10^2	96.5 %
30 kg washed lettuce #1 " " " " #2 " " " " #3	2.64×10^3 8.13×10^2 1.74×10^3	1.39×10^3	92.5 %

* colony forming units per gram (cfu/g)

4. Discussion

Typically the total microbial count on fresh-cut samples was reduced by 90 to 99%. A substantial microbial reduction (>90%) was achieved using POAA levels even at 5 ppm. Batch washing trial demonstrated a comparable washing performance between Tsunami™ and free chlorine at equivalent concentrations, with respect to microbial reduction on shredded lettuce and the depletion of each sanitiser during the washing trials.

Contact time trials demonstrated that a substantial (>90%) microbial reduction was achieved with spraying or a 30 second immersion of shredded lettuce. Therefore it is suitable for applications where there is a short-term exposure of the product either by spraying or fast moving flumes. However prolonged exposure in an immersion tank is advisable to ensure adequate mixing of the product, such that all surfaces become exposed, especially if the raw product is very dirty.

The Tsunami™ product was very convenient to use, being applied in the same manner as chlorine from a concentrated stock solution. The washing bath can be dosed manually or by a pump system that is controlled via milli-volt readings from an oxidative redox potential (ORP) probe. The later method is designed to maintain POAA levels in a continuous water recycling washing systems and reduce worker exposure. The ORP monitoring/controller system shall be investigated in further trials.

Can be easily monitored with on the spot checks using POAA test strips by a process operator, taking less than 1 minute to conduct. Intermittent determination of POAA levels is recommended as this is one of the critical control points for a HACCP system.

Tsunami™ breaks down to acetic acid, water and oxygen. With use of test strip, on the surface of washed lettuce, could not detect any POAA (LOD=5ppm) after 1 hour when prior to microbial sampling. Therefore this product can be used without requiring a final water rinse, as presently used for chlorine operations.

5. Key Findings

* Generally the total viable population on fresh-cut samples was reduced within the range of 90% to 99%.

* Tsunami™ very convenient to use.

* Washing performance validated using chilled water of 2-3°C.

* Ease of monitoring with POAA test strips

* No requirement for final water rinse after Tsunami™ treatment.

FURTHER WORK

* Evaluation of continuous washing system controlled by ORP monitoring and controlling of POAA levels.

* Further residue testing and sensory evaluation.

Milestone #4: Pilot-scale washing trials on minimally processed vegetables using a mixed oxidant system

EXECUTIVE SUMMARY

As a participant of the CRC-IFMPS, Food Science Australia has conducted evaluations on 4 alternatives to chlorine sanitising washing systems. These have included (i) MCV-resin (iodine), (ii) Tsunami™ (peracetic acid), (iii) silver / hydrogen peroxide system and (iv) the mixed oxidant system. The evaluation of the mixed oxidant system was supported by the HRDC.

Pilot-scale trials were undertaken to determine the suitability of using mixed oxidants (MIOX) developed for water treatment by the US military, for the washing of minimally processed vegetables. The MIOX unit was hired from Contamination Control Ltd. in New Zealand, as this system was not distributed in Australia. The MIOX system produces hypochlorous acid (100-200 ppm), chlorine dioxide (10-20 ppm) and ozone (1-2 ppm) in a water stream, by passing a salt solution through an electrolytic cell.

Contact time studies demonstrated that the reduction in viable count was more effective when the product was immersed in the MIOX solution (minimum of 30 seconds), rather than spraying onto the product. An extended immersion time did not provide significant additional microbial reduction. Generally the contact time used in industry is longer (1-2 minutes) to ensure proper mixing and exposure to all surfaces.

The pilot-scale washing trials generally achieved a reduction in the total viable count on shredded lettuce of 90 to 99% (1-2 log counts). During the batch washing trial the % microbial reduction declined to less than 90% with a corresponding reduction in the level of free chlorine, necessitating the re-dosing of the washing bath with fresh MIOX treated water. Chilled water of 2-3°C was used which is favourable as chilling reduces produce respiration rates and microbial growth.

MIOX was more effective in reducing the viable numbers of *E.coli* on the lettuce surface, followed by *Salmonella*, and least effective against *Listeria monocytogens* . For shredded lettuce artificially inoculated with *Listeria monocytogenes, Salmonella,* and *E.coli* the viable numbers were reduced by 89.6 %, 70.0 % and 93.1 % respectively by washing with 60 ppm free chlorine, and by 86.5 %, 94.8 % and 95.7 % respectively using 120 ppm free chlorine. The challenge trial demonstrated that a free chlorine level of 60 ppm was not sufficient (ie. >90% microbial reduction) for *Listeria monocytogenes* and *Salmonella*.

The MIOX system can be purchased to treat large quantities of water, well in excess of that required by even the largest fresh-cut operations in Australia. As the oxidants are rapidly depleted and the concentration of oxidants generated by the MIOX system is comparable to the levels of chlorine currently used by processors then the water must be continuously replenished, such as in a flow-through wash tank, in order to maintain satisfactory produce sanitation. This would result in the use of large quantites of water and pose problems associated with disposal and treatment of waste washing water.

1. Aim

To determine the suitability of a Mixed Oxidant (MIOX) system for the sanitation of fresh-cut vegetables by pilot-scale studies, including the establishment of operating conditions and investigating the effectiveness of pathogen reduction.

2. Methodology

2.1 Operational procedure for washing trials

2.1.1 Operational set up of washing system

A solution contaning hypochlorous acid, chlorine dioxide and ozone was generated using a MIOX system supplied by Contamination Control. A salt solution was pumped through an electrolytic cell (operating at 6 amps) to generate hypochlorous acid (100-200 ppm), chlorine dioxide (10-20 ppm) and ozone (0.5-1.0 ppm) at a preset ratio. The resultant MIOX solution was pumped directly into the washing bath and diluted with tap water if necessary to achieve the desired oxidant concentration. Free chlorine level, the major oxidant species was monitored using a chlorine microprocesser HI93711 (Hanna Instruments), and a n,n-diethyl-p-phenylenediamine (DPD) reagent. The MIOX solution was cooled to 2-3 °C (by recycling through a plate heat exchanger), pH was adjusted to 6.0-6.5 using 2% citric acid, and free chlorine levels were checked prior to washing the produce.

2.1.2 Summary of operational parameters

Flow rate of recycling water: 700 litres/hour
Product immersion time of 3 minutes (conveyor speed setting of 0.5)
Continual operation of a spray fan jet (200 litres/hour) and 5 air jets.
Volume of washing bath: 60 litres
Volume of overfill tank: 40 litres
Total volume of water in recycling system: 120 litres
Filters: 2 mm screen, 150 μm basket filter, 10 μm filter
Pressure: 120 kPa
pH: 6.0-7.0
Product load rate: about 5 kg / 6 minutes
Temperature of water in washing bath 2-3°C

Note: These are the standard operating parameters except where otherwise specified.

2.2 Product preparation

Shredded lettuce:
Iceberg lettuce was purchased the day before processing and stored overnight at 2°C. The outer leaves and stem were removed, the lettuce trimmed of any browned or soiled sections, the heads were halved and the core removed, and finally the product was shredded into 1 cm wide strips.

Broccoli florets:
Broccoli heads were purchased the day before processing and stored overnight at 2°C. Small florets of about 2-4 cm in diameter were cut from the main stem, suitable for inclusion in vegetable medleys and stir-fries. The main stem was discarded.

Carrot batons:
Carrots were topped and tailed then cut to 6 cm lengths, then abrasively peeled and rinsed with tap water to remove peeled particles.

2.3 Washing, spin drying and packaging

Product was placed on a conveyor which travelled trough the washing bath. Product was pre-sprayed then immersed for 3 minutes. Washed product was spin-dried, then weighed and packaged into permeable polypropylene pouches and sealed using a Freshline packaging machine.

2.4 Product Testing

2.4.1 Microbiological analysis

Products were sampled in triplicate for microbiological analysis, 20 gram portions were homogenised (Colworth Stomacher) in 180 mL of 0.1 % bacteriological peptone (Oxoid), then spiral plated on plate count agar (Oxoid) and incubated for 2 days at 30°C.

Pathogen study:
Shredded lettuce was inoculated with either *Salmonella salford, Listeria monocytogens* or *Escherichia coli*, using a dipping solution. Inoculated lettuce was stored for 5 hours at 10°C, prior to washing with MIOX treated water. Products were sampled in triplicate for microbiological analysis, 20 gram portions were homogenised (Stomacher) in 180 mL of 0.1 % bacteriological peptone (Oxoid), then pour plated using XLD (Oxiod) for *Salmonella salford*, incubated at 37°C for 1 day; Listeria selective agar (Oxiod) for *Listeria monocytogens*, incubated at 30°C for 1 day; and Chromocult agar (Merck) for *Escherichia* incubated at 37°C for 1 day.

2.4.2 Statistical Analysis

Mean of 3 replicate determinations are presented with respective standard deviation. Analysis of variance (single factor) was calculated using Genstat version 3.2 for calculation of least significant difference ($LSD_{0.95}$). Difference between mean values greater then $LSD_{0.95}$ is significant, at $p<0.05$.

3. Results

3.1 Effect of contact time (spraying and dipping) on washing performance

The washing contact time was investigated to determine the effects on viable count of the product. The shortest contact time was achieved by a single fan-spray which equated to about a 6 second contact period. Longer contact periods were achieved by the immersion of the lettuce in the MIOX treated water for periods of 30, 60 and 120 seconds. The free chlorine level was approximately 20 ppm.

Spraying of the product only resulted in a microbial reduction of 82% (see Table 1), most probably due to inadequate sanitiser contact with product surfaces. In contrast, immersion of the lettuces resulted in a more favourable microbial reduction. The microbial population of the unwashed lettuce control was significantly different ($p<0.05$) to all the immersion treatments but not the spraying treatment. The spraying treatment was significantly different ($p<0.05$) to an immersion treatment of 30 seconds.

Table 1. Effect of contact time on the microbiological quality of shredded lettuce washed with MIOX treated water.

Lettuce Treatment with MIOX-contact time	Mean* ($Log_{10}cfu/g$)	% Reduction in cfu/g
Unwashed (control)	4.38 ± 0.69	—
Spray wash (6 seconds)	3.76 ± 0.32	82.0
Immersion for 30 seconds	3.09 ± 0.30	96.6
Immersion for 1 minute	3.26 ± 0.05	95.5
Immersion for 3 minutes	3.62 ± 0.12	89.6

* Difference between mean values greater than $LSD_{0.95}$ of 0.68 is significant ($p<0.05$).

3.2 Washing of shredded lettuce, carrot batons and broccoli florets.

The total viable count for shredded lettuce, broccoli florets and carrot batons washed (immersed for 3 minutes) with MIOX treated water at free chlorine concentrations of 76.5 ppm, 82.5 ppm and 77.5 ppm respectively, was reduced by 99.5 %, 90.5 % and 78.1% respectively (see Tables 2, 3,and 4). The microbial reduction for the carrot batons was lower because the batons were prewashed in water to remove any residual peel. The total microbial population on MIOX washing product was significantly different ($p<0.05$) to unwashed product, for shredded lettuce, carrot batons and broccoli florets.

Table 2. Total viable count of washed shredded lettuce

Shredded lettuce treatment	Mean* ($Log_{10}cfu/g$)	% Reduction in cfu/g
Unwashed (control)	4.90 ± 0.68	—
MIOX washed	2.95 ± 0.38	99.5

* Difference between mean values greater than $LSD_{0.95}$ of 0.44 is significant ($p<0.05$).

Table 3. Total viable count of washed broccoli florets

Broccoli floret treatment	Mean* (Log_{10}cfu/g)	% Reduction in cfu/g
Unwashed (control)	6.43 $\pm$ 0.08	—
MIOX washed	5.36 $\pm$ 0.26	90.5

* Difference between mean values greater than $LSD_{0.95}$ of 0.44 is significant ($p<0.05$).

Table 4. Total viable count of washed carrot batons

Carrot baton treatment	Mean* (Log_{10}cfu/g)	% Reduction in cfu/g
Unwashed (control)	4.83 $\pm$ 0.17	—
MIOX washed	4.18 $\pm$ 0.10	78.1

* Difference between mean values greater than $LSD_{0.95}$ of 0.44 is significant ($p<0.05$).

3.3 Effect of washing on pathogens, *Listeria monocytogenes, Salmonella salford,* and *Escherichia coli.*

MIOX treated water reduced populations of *Listeria monocytogenes, Salmonella salford, Escherichia coli* on artificially contaminated shredded lettuce. The levels of *Listeria monocytogenes, Salmonella salford, Escherichia coli* were reduced by 89.6 %, 70.0% and 93.1% using 60 ppm free chlorine, and by 86.5 %, 94.8% and 95.7% using 120 ppm free chlorine.

***Escherichia coli* challenge trial:**

Shredded lettuce was inoculated with *Escherichia coli* at about 7.34x 10^6 cfu/g. The level of inoculation is considered high with fresh-cut product. The shredded lettuce was washed with MIOX treated water at free chlorine concentrations of 60 ppm and 120 ppm. Following washing of the inoculated shredded lettuce the *Escherichia coli* population was reduced by 93.1 % and 95.7 % using 60 and 120 ppm free chlorine respectively (Table 5). The use of MIOX treated water at 120 ppm free chlorine was significantly ($p<0.05$) more effective then using MIOX at 60ppm free chlorine.

Table 5. Summary of *E.coli* challenge trial results

Lettuce Treatment	Mean* (Log_{10}cfu/g)	% Reduction in cfu/g
Unwashed (blank)	<1.00 $\pm$ 0.00	—
E.coli innoculated (control)	6.86 $\pm$ 0.07	—
MIOX free chlorine 60ppm	5.70 $\pm$ 0.05	93.1
MIOX free chlorine 120ppm	5.49 $\pm$ 0.08	95.7

* Difference between mean values greater than $LSD_{0.95}$ of 0.14 is significant ($p<0.05$).

***Salmonella spp.* challenge trial:**

Shredded lettuce was inoculated with *Salmonella spp* at about 3.87 x 10^2 cfu/g. The shredded lettuce was washed with MIOX treated water at free chlorine concentrations of 60 ppm and 120 ppm. Following washing of the inoculated shredded lettuce the *Salmonella salford* population was reduced by 70.0 % and 94.8 % using 60 and 120 ppm of free chlorine respectively (Table 6). There was no significant difference between *Salmonella* populations between the treatments, as a result of a large variance in small populations.

Table 6. Summary of *Salmonella* challenge trial results

Lettuce Treatment	Mean* (Log_{10}cfu/g)	% Reduction in cfu/g
Unwashed (blank)	<1.00 $\pm$ 0.00	—
Salmonella innoculated (control)	2.19 $\pm$ 0.73	—
MIOX free chlorine 60ppm	1.92 $\pm$ 0.45	70.0
MIOX free chlorine 120ppm	1.26 $\pm$ 0.24	94.8

* Difference between mean values greater than $LSD_{0.95}$ of 1.03 is significant ($p<0.05$).

***Listeria monocytogenes* challenge trial:**

Shredded lettuce was inoculated with *Listeria monocytogenes* at about 1.11 x 10^6 cfu/g. The level of inoculation is considered high with fresh-cut product. The shredded lettuce was washed with MIOX treated water at free chlorine concentrations of 30 ppm 60 ppm and 120 ppm. Following washing of the inoculated shredded lettuce the *Listeria monocytogenes* population was reduced by 64.8 %, 89.6 % and 86.5 % using 30, 60 and 120 ppm of free chlorine respectively (Table 7). The population of *L.monocytogenes* in MIOX washed samples was significantly different to the innoculated control, but there was no significant difference between MIOX washing levels.

Table 7. Summary of *L.monocytogenes* challenge trial results

Lettuce Treatment	Mean* (Log_{10}cfu/g)	% Reduction in cfu/g
Unwashed (blank)	<1.00 $\pm$ 0.00	—
L.monocytogenes innoculated (control)	6.39 $\pm$ 0.51	—
MIOX free chlorine 30ppm	5.49 $\pm$ 0.38	64.8
MIOX free chlorine 60ppm	5.04 $\pm$ 0.15	89.6
MIOX free chlorine 120ppm	5.17 $\pm$ 0.01	86.5

* Difference between mean values greater than $LSD_{0.95}$ of 0.68 is significant ($p<0.05$).

3.5 MIOX batch washing trial.

A batch washing trial was performed with shredded lettuce to determine washing performance with increasing organic load in the washing bath. Washing performance indicators included the measurement of free chlorine concentration, water quality by ATP-bioluminescence and microbial count, and the microbial quality of the product. These tests were performed and samples collected at 5 kg intervals of washed shredded lettuce for a total of 20 kg.

At commencement of the batch washing trial the free chlorine concentration was 77 ppm and this level declined rapidly throughout the trial (Table 8). Following the washing of 20 kg of shredded lettuce the free chlorine concentration was reduced by 64 %. ATP levels increased rapidly during the batch washing trial (Table 8), indicating the build up of organic matter in the washing tank.

Table 8. Chlorine levels and water quality during shredded lettuce batch washing trial

Product load	Free Chlorine (ppm)	Water ATP (RLU)
Prewash	77	35
After 5 kg	70	2685
After 10kg	58	4211
After 15 kg	34	13611
After 20 kg	28	17392
After 21 kg	24	17631

For the initial 5 kg of shredded lettuce that was washed, a very favourable microbial reduction of 99.5 % was achieved. However this was not maintaind as subsequent washing intervals did not achieve even 90% microbial reduction. This maybe due to the rapid depletion of sanitisers, as illustrated for free chlorine in Table 9. Although chlorine dioxide and ozone were not tested their depletion would be particulalry important factor in reducing individual reduction efficiancy.

Table 9. Microbial quality of shredded lettuce from MIOX batch washing trial

Lettuce Treatment	Mean* (Log_{10}cfu/g)	% Reduction in cfu/g
Unwashed (control)	4.90 ± 0.68	—
5 kg washed lettuce	2.95 ± 0.38	99.9
10 kg washed lettuce	4.98 ± 0.34	54.0
15 kg washed lettuce	4.25 ± 0.51	87.9
20 kg washed lettuce	3.88 ± 0.05	89.6

* Difference between mean values greater than $LSD_{0.95}$ of 0.81 is significant ($p<0.05$).

4. Discussion

Typically the total microbial count on fresh-cut samples was reduced by 90 to 99%. Preliminary trials (April-June 1997) demonstrated that MIOX treated water provided a more effective sanitation to equivalent concentrations of chlorine.

Contact time trials demonstrated that an adequate microbial reduction (of >90%) was not achieved with spraying. Immersion of shredded lettuce for 30 seconds or more provided greater microbial reduction. Therefore it is recommended that MIOX is more suitably applied by immersion of the product, even if only for a short period (ie 30 seconds) such as in a fast moving flume. However prolonged exposure in an immersion tank may be advisable to ensure adequate mixing of the product, such that all surfaces become exposed, especially if the raw product is very dirty.

Once the MIOX solution has been generated it must be used rapidly, because the oxidants gas off. MIOX systems can be configured to operate in a continuous manner to treat large volumes of water. The washing bath can be dosed manually or by a pump system that is controlled via milli-volt readings from an oxidative redox potential (ORP) probe. The later method is designed to maintain oxidant levels in a continuous water recycling washing systems and reduce worker exposure. When treating smaller volumes of water, particularly for batch washing operations it is not as practical as using a concentrate such as chlorine.

Each of the oxidants can be easily monitored using colorimetric analytical kits (ie Lovibond system supplied by Labsupply). Intermittent determination of sanitiser levels is recommended as this is one of the critical control points in a HACCP system.

The level of chlorinated residues (ie. trihalomethanes) are reduced when using MIOX, compared with current practice of chlorine washing. Thereby minimising the risk to workers, consumers and the environment.

5. Key Findings

* Generally the total viable population on fresh-cut samples was reduced within the range of 90% to 99%.

* Monitoring can be performed using the same systems as currently used for chlorine

* Application of MIOX requires final water rinse as currently used following chlorine washing.

Milestone #5: Pilot-scale washing trials on minimally processed vegetables using silver hydrogen peroxide system

EXECUTIVE SUMMARY

Pilot-scale trials were undertaken to determine the suitability of using a silver / hydrogen peroxide system for the washing of minimally processed vegetables. This system was developed by Austech P.L. for the disinfection of water. The combined effect of silver and hydrogen peroxide is claimed to provide an enhanced microbiological kill, furthermore the silver ions are claimed to provide a residual antimicrobial activity during water storage. This system is distributed by Homepure International P.L.. For pilot-scale trials a system was borrowed from Melbourne University, Gilbert Chandler Campus.

The CRC sought to perform an extensive evaluation to determine the effectivness of the silver / hydrogen peroxide system in improving microbiological quality of the product, including challenge trials with human pathogens onto artificially inoculated shredded lettuce. The silver system was operated at 20 and 28 mAmp which resulted in the released of silver ion in the range of 0.8 to 1.1 ppm. The silver treated water was dosed with 0.04% hydrogen peroxide.

Contact time studies indicated that immersion fro 1 to 3 mins provided a margianlly more favourable microbial reduction then contact for only 30 seconds or spraying. Generally the contact time used in industry is longer (1-2 minutes) to ensure proper mixing and exposure to all surfaces. The pilot-scale washing trials achieved a reduction in the total viable count on shredded lettuce in the range of 25% to 85%. For shredded lettuce artificially inoculated with *Listeria monocytogenes, Salmonella spp.,* and *E.coli* the viable numbers were reduced by 5.4%, 48% and 23% respectively by washing with 1.0 ppm silver and 0.04% hydrogen peroxide. The microbial reduction was unsatisfactory for all of the washing trials (ie. less than 90%).

The silver residue levels on shredded lettuce and broccoli florets are comparable with the maximum residue limit of silver for drinking water in the ANZFA-Food Standards Code. Therefore increasing of the silver levels to improve product sanitation is likely to produce unacceptably high level of silver residue.

1. Aim

To determine the suitability of a silver/hydrogen peroxide system for the sanitation of fresh-cut vegetables using pilot-scale trials; including establishment of appropriate washing system design, operating conditions and investigating effectiveness against targetted pathogens.

2. Methodology

2.1 Operational procedure for washing trials

2.1.1 Operational set up of washing system

The silver system consisted of two individual electrodes contained in seperate cannitsters situated in series, through which town water flowed. The electrodes operated at a setting of 20 or 28 mAmps, and a flow rate of approximately 80 liters per hour The silver treated water was added directly to the washing bath then hydrogen peroxide was added to a final concentration of 0.04%. The water was then cooled to 2-3°C prior to washing the produce. The pH was adjusted to approximately 7.5, with 2% citric acid.

2.1.2 Summary of operational parameters

Flow rate of recycling water: 700 litres/hour
Product immersion time of 3 minutes (conveyor speed setting of 0.5)
Continual operation of a spray fan jet (200 litres/hour) and 5 air jets.
Volume of washing bath: 60 litres
Volume of overfill tank: 40 litres
Total volume of water in recycling system: 120 litres
Filters: 2 mm screen, 150 μm basket filter, 10 μm filter
Pressure: 120 kPa
pH: 7.5
Product load rate: about 5 kg / 6 minutes
Temperature of water in washing bath 2-3°C

Note: These are the standard operating parameters except where otherwise specified.

2.2 Product preparation

Shredded lettuce:
Iceberg lettuce was purchased the day before processing and stored overnight at 2°C. The outer leaves and stem were removed, then trimmed of any browned or soiled sections, the heads were halved and the core removed, then shredded into 1 cm wide strips.

Broccoli florets:
Broccoli heads were purchased the day before processing and stored overnight at 2°C. Small florets of about 2-4 cm in diameter were cut from the main stem, suitable for inclusion in vegetable medleys and stir-fries. The main stem was discarded.

Carrot batons:
Carrots were topped and tailed then cut to 6 cm lengths, then abrasively peeled and rinsed with tap water to remove peeled particles.

2.3 Washing, spin drying and packaging

Product was placed on a conveyor which travelled trough the washing bath. Product was pre-sprayed then immersed for 3 minutes. Washed product was spin-dried, then weighed and packaged into permeable polypropylene pouches and sealed using a freshline packaging machine.

2.4 Product Testing

2.4.1 Microbiological analysis

Products were sampled in triplicate for microbiological analysis, 20 grams portions were homogenised (Colworth Stomacher) in 180 mL of 0.1 % bacteriological peptone (Oxoid), then spiral plated on plate count agar (Oxoid) and incubated for 2 days at 30°C.

Pathogen study:
Shredded lettuce was inoculated with either *Salmonella spp, Listeria monocytogens* or *Escherichia spp*, using a dipping solution. Inoculated lettuce was then stored for 5 hours at 10°C, then washed using silver/hydrogen peroxide treated water. Products were sampled in triplicate for microbiological analysis, 20 grams portions were homogenised (Stomacher) in 180 mL of 0.1 % bacteriological peptone (Oxoid), then pour plated using XLD (Oxiod) for *Salmonella spp*, incubated at 37°C for 1 day; Listeria selective agar (Oxiod) for *Listeria monocytogens*, incubated at 30°C for 1 day; and Chromocult agar (Merck) for *Escherichia spp* incubated at 37°C for 1 day.

2.5 Residue testing for silver levels in water and product.

Residue testing for silver levels was performed using atomic absorption spectrometry at State Chemistry Laboratories.

3. Results

3.1 Effect of contact time (spraying and dipping) on washing performance

The washing contact time was investigated to determine what effect it had on the reduction of microbial populations. The shortest contact time was attained by a single fan-spray which equated to about a 6 second contact period. Longer contact periods were attained by the immersion of the lettuce in the silver (1.0 ppm at 20 mAmps) /hydrogen peroxide (0.04%) treated water for periods of 30, 60 and 120 seconds.

For all of the treatments the microbial reduction was unsatisfactory (ie. less than 90% total microbial reduction). However the contact trial indicated that prolonged immersion of the product for 1 to 3 minutes resulted in a marginally better microbial reduction (Table 1). A longer immersion time increases the likelihood of exposure to all surfaces provided sufficient mixing occurs.

Table 1. Effect of contact time on the microbiological qualty of shredded lettuce washed with silver / hydrogen peroxide treated water.

Lettuce Treatment with silver / hydrogen peroxide: -contact time	**Total Count (cfu/g)***	**Average (cfu/g)**	**% Microbial reduction for each contact time**
Unwashed spray control #1 " " " #2 " " " #3	4.63×10^4 3.98×10^4 6.53×10^4	5.05×10^4	—
Spray wash (6 seconds) #1 " " " #2 " " " #3	1.23×10^4 9.55×10^3 1.77×10^4	1.32×10^4	73.2 %
Immersion for 30 seconds-control #1 " " " " " #2 " " " " " #3	1.12×10^5 3.74×10^4 4.67×10^4	6.54×10^4	—
Immersion for 30 seconds #1 " " " " #2 " " " " #3	1.99×10^4 9.15×10^3 1.28×10^4	1.40×10^4	78.6 %
Immersion for 1 minute-control #1 " " " " " #2 " " " " " #3	3.82×10^4 4.88×10^4 1.70×10^5	8.57×10^4	—
Immersion for 1 minute #1 " " " " #2 " " " " #3	9.35×10^3 1.52×10^4 1.69×10^4	1.38×10^3	83.4 %
Immersion for 3 minutes control #1 " " " " " #2 " " " " " #3	3.11×10^5 2.46×10^5 Not tested	2.79×10^5	—
Immersion for 3 minutes #1 " " " " #2 " " " " #3	4.96×10^4 5.04×10^4 5.73×10^4	5.24×10^4	81.2 %

3.2 Washing of shredded lettuce, carrot batons and broccoli florets.

The total viable count for shredded lettuce and broccoli florets washed with silver (1.1 and 0.83 ppm respectively, at 20 mamps) / hydrogen peroxide (0.04 %) treated water, was reduced by 73.3 % and 69.8 % (Tables 2, 3). The microbial count for the carrot batons increased (250 %) as compared with abrasively peeled and water washed carrot battons (Table 4). There was considerable variation in the carrot counts which maybe attributed to the presence of any residual peel, which has a much higher microbial count then the underlying tissue.

Table 2. Total viable count of washed shredded lettuce

Samples	Unwashed lettuce (cfu/g)*	Washed lettuce (cfu/g)
1	4.63×10^4	1.23×10^4
2	3.98×10^4	9.55×10^3
3	6.53×10^4	1.77×10^4
Average	**5.05×10^4**	**1.32×10^4**

*colony forming units per gram (cfu/g)

Table 3. Total viable count of washed broccoli florets

Samples	Unwashed broccoli (cfu/g)*	Washed broccoli (cfu/g)
1	1.78×10^6	2.20×10^5
2	2.26×10^6	8.53×10^4
3	9.07×10^5	1.19×10^6
Average	**1.64×10^6**	**4.98×10^5**

*colony forming units per gram (cfu/g)

Table 4. Total viable count of washed carrot batons

Samples	Peeled & water washed carrots (cfu/g)*	Washed carrots (cfu/g)
1	2.03×10^3	6.10×10^2
2	5.08×10^3	2.11×10^4
3	1.62×10^3	3.05×10^3
Average	**2.91×10^3**	**8.25×10^3**

*colony forming units per gram (cfu/g)

3.3 Effect of washing on pathogens, *Listeria monocytogenes, Salmonella spp.*, and *Escherichia coli.*

Silver (1.0 ppm, at 20 mAmps) / hydrogen peroxide (0.04 %) treated water reduced populations of *Listeria monocytogenes, Salmonella salford, Escherichia coli* on artificially contaminated shredded lettuce. The levels of *Listeria monocytogenes, Salmonella spp., Escherichia coli* were reduced by 5 %, 48 % and 74 % (Table 5,6 & 7). For all treatments the % of microbial reduction was unsatisfactory (ie. less than 90%).

Table 5. Summary of *E.coli* challenge trial results

Lettuce Treatment	E.coli (cfu/g)*	Average (cfu/g)	% Reduction
Unwashed (blank) #1	$< 1 \times 10^1$		
" " #2	$< 1 \times 10^1$	$< 1 \times 10^1$	—
" " #3	$< 1 \times 10^1$		
E.coli innoculated (control) #1	1.77×10^6		
" " " #2	4.60×10^5	1.03×10^6	—
" " " #3	8.72×10^5		
E.coli Ag^+ / H_2O_2 washed #1	2.56×10^5		
" " " " #2	2.68×10^5	2.65×10^5	74 %
" " " " #3	2.72×10^5		

* colony forming units per gram (cfu/g)

Table 6. Summary of *Salmonella* challenge trial results

Lettuce Treatment	Salmonella (cfu/g)*	Average (cfu/g)	% Reduction
Unwashed (blank) #1	$< 1 \times 10^1$		
" " #2	$< 1 \times 10^1$	$< 1 \times 10^1$	—
" " #3	$< 1 \times 10^1$		
Salmonella innoculated (control) #1	2.07×10^3		
" " " " #2	2.23×10^3	2.03×10^3	—
" " " " #3	1.80×10^3		
Salmonella Ag^+ / H_2O_2 washed #1	1.13×10^3		
" " " " #2	1.03×10^3	1.06×10^3	48 %
" " " " #3	1.01×10^3		

* colony forming units per gram (cfu/g)

Table 7. Summary of *Listeria monocytogenes* challenge trial results

Lettuce Treatment	L.monocytogenes (cfu/g)*	Average (cfu/g)	% Reduction
Unwashed (blank) #1	$< 1 \times 10^1$		
" " #2	$< 1 \times 10^1$	$< 1 \times 10^1$	—
" " #3	$< 1 \times 10^1$		
L.monocytogenes innoculated (control) #1	4.80×10^5		
" " " " " #2	1.34×10^6	8.63×10^5	—
" " " " " #3	7.68×10^5		
L.mono. E.coli Ag^+ / H_2O_2 washed #1	4.85×10^5		
" " " " #2	2.68×10^6	8.16×10^5	5 %
" " " " #3	7.04×10^5		

3.4 Batch washing trial: a comparison between Tsunami™ and chlorine washing

A batch washing trial was performed with shredded lettuce to determine washing performance with increasing organic load in the washing bath. Washing performance indicators included the measurement of silver concentration, water quality by ATP-bioluminescence and microbial count, and the microbial quality of the product. These tests were performed and samples collected at 5 kg intervals of washed shredded lettuce for a total of 30 kg. Initially, the silver level was 1.0 ppm, produced at 20 mAmps) and hydrogen peroxide (0.04 %).

The organic levels in the washing bath rapidly increased throughout the batch washing trial. Correspondingly, the total number of bacteria in the washing water increased to approximately 10,000 cfu/100mL (Table 8).

The silver levels in the washing water did not demonstrate a utilisation of the silver ions by attachment to the product and subsequent removal from the washing water (Table 8).

Throughout the batch washing trial the % microbial reduction was unsatisfactory (ie. less than 90 %). (Table 9)

Table 8. Summary of water testing during batch washing trial.

Wash water sampling intervals	ATP-bioluminesence (RLU)	Silver[1] (ppm)	Total Mesophillic Count[2] (cfu/100mL)
Prewash	34	0.83	2
After 5kg of lettuce	4231	1.31	13
After 10kg of lettuce	10271	0.99	3360
After 15kg of lettuce	19852	1.00	4720
After 20kg of lettuce	32015	1.15	10800
After 25kg of lettuce	36148	1.05	11800
After 30kg of lettuce	37890	0.92	10800

[1] average of duplicate water samples

[2] Average of triplicate sampling

Figure 9. Microbial quality of shredded lettuce from silver / hydrogen peroxide batch washing trial

Lettuce Treatments	Total count (cfu/g)	Average (cfu/g)	% Reduction
5 kg Unwashed lettuce-control #1	1.20×10^6		
" " " " #2	1.28×10^6	1.13×10^6	—
" " " " #3	1.00×10^6		
5 kg washed lettuce #1	4.44×10^5		
" " " " #2	4.63×10^5	4.81×10^5	57 %
" " " " #3	5.37×10^5		
10 kg Unwashed lettuce-control #1	8.89×10^5		
" " " " #2	1.19×10^6	9.83×10^5	—
" " " " #3	8.70×10^5		
10 kg washed lettuce #1	7.78×10^5		
" " " " #2	5.56×10^5	6.55×10^5	33 %
" " " " #3	6.30×10^5		
15 kg Unwashed lettuce-control #1	1.17×10^6		
" " " " #2	1.07×10^6	1.17×10^6	—
" " " " #3	1.28×10^6		
15 kg washed lettuce #1	5.56×10^5		
" " " " #2	5.56×10^5	6.36×10^5	46 %
" " " " #3	7.96×10^5		
20 kg Unwashed lettuce-control #1	1.15×10^6		
" " " " #2	1.56×10^6	1.13×10^6	—
" " " " #3	6.67×10^5		
20 kg washed lettuce #1	9.26×10^5		
" " " " #2	6.30×10^5	7.53×10^5	33 %
" " " " #3	7.04×10^5		
25 kg Unwashed lettuce-control #1	7.96×10^5		
" " " " #2	1.37×10^6	1.01×10^6	—
" " " " #3	8.52×10^5		
25 kg washed lettuce #1	7.78×10^5		
" " " " #2	8.52×10^5	7.59×10^5	25 %
" " " " #3	6.48×10^5		
30 kg Unwashed lettuce-control #1	1.24×10^6		
" " " " #2	9.07×10^5	1.02×10^6	—
" " " " #3	9.26×10^5		
30 kg washed lettuce #1	1.63×10^5		
" " " " #2	4.26×10^5	4.74×10^5	54 %
" " " " #3	8.33×10^5		

3.5 Residue testing for silver levels on minimally processed vegetables.

The silver residue levels of unwashed minimally processed vegetables was less than the detection limit of 0.02 ppm. Following washing of baby carrots in silver treated water (0.83 ppm), silver ws not detected (Table 11). When broccoli florets and shredded lettuce were washed in silver treated water at 0.83 ppm and 1.1 ppm respectively, the average silver level on each product was found to be 0.09 ppm and 0.10 ppm (table 10 and 12). The silver residue levels on shredded lettuce and broccoli florets are comparable with the maximum residue limit of silver for drinking water in the ANZFA-Food Standards Code.

Table 10. Silver levels on broccoli florets washed with 0.83 ppm Ag^+

Samples	Unwashed broccoli florets (ppm)	Washed broccoli florets(ppm))
1	< 0.02	0.07
2	< 0.02	0.11
Average	**< 0.02**	**0.09**

Table 11. Silver levels on baby carrots washed with 0.99 ppm Ag^+

Samples	Unwashed baby carrots (ppm)	Washed baby carrots (ppm))
1	< 0.02	< 0.02
2	< 0.02	< 0.02
Average	**< 0.02**	**< 0.02**

Table 12. Silver levels on shredded lettuce washed with 1.1 ppm Ag^+

Samples	Unwashed shredded lettuce (ppm)	Washed shredded lettuce (ppm)
1	< 0.02	0.11
2	< 0.02	0.10
3	< 0.02	0.11
4	< 0.02	0.12
5	< 0.02	0.11
6	< 0.02	0.11
7	< 0.02	0.07
8	< 0.02	0.06
Average	**< 0.02**	**0.10**

4. Discussion

For all of the washing trials the microbial reduction was unsatisfactory, using silver treated water in the range of 0.8 to 1.1 ppm. The concentration of silver could be increased by using a slower flow rate, therefore increasing the residence time of the water within the silver electrode chambers. However, when using a slower flow rate the time required to fill a washing bath would be unpractical. The system was operated at 28mAmps per electrode which is the recomended maximum setting. Consequently, a larger system electrode system would need to be constructed.

The silver residue levels on shreddded lettuce was already comparable with the maximum used for drinking water in the ANZFA-Food Standards Code, this indicates that using higher levels of silver to improve the sanitation of produce is likely to result in unacceptably high silver residual levels.

Milestone #6: Cost-benefit analysis for chlorine alternatives for washing of minimally processed vegetables

EXECUTIVE SUMMARY

This report was designed to provide a cost-benefit analysis of the chlorine alternatives based upon: (i) microbiological criteria, (ii) operating and capital cost, (iii) ease of integration into existing washing practices, (iv) monitoring of sanitiser levels for a food safety program, (v) registration (ANZFA) and residue issues.

Pilot scale fresh-cut vegetable processing trials had been conducted and 4 potential chlorine alternatives have been evaluated for the purpose of replacing chlorine as the sanitising agent in the produce washing system. These included, (i) MCV-resin system (iodine), (ii) Mixed oxidant system (HOCl, ClO_2, O_3), (iii) Tsunami™ (peracetic acid) and a (iv) Silver / hydrogen peroxide system.

Overall Tsunami was the most effective chlorine alternative based on the microbiological criteria, of total microbial load and reduction of pathogens of concern to food safety. In addition, Tsunami can be conveniently introduced into working systems in a similar way to chlorine, applicable for spraying, fast flumes and washing tanks.

The MCV resin system was also a favourable alternative as it is none corrosive and convenient to use. The system presently marketed in Australia has limitations relating to amount of released iodine and higher water temperatures required for release of iodine (necessitating the use of a heat exchanger for optimal operation).

The mixed oxidant system and the silver / hydrogen peroxide system did not satisfy functional requirements, particularly if used in a recycling washing system. Was also rejected on microbiological criteria (ie. poor sanitation performance) and in addition the MIOX system only offers a partial reduction in the level of harmful by-products.

Therefore the Tsunami product was recommended for industry adoption as a chlorine alternative for washing fresh-cut vegetables. However, this recomendation is subject to performance of trials under commercial processing conditions. Further work is required to validate a continuous monitoring and dosing system, suitable for larger processing operations.

1. Introduction

Pilot-scale fresh-cut vegetable processing trials have been conducted and 4 potential chlorine alternatives evaluated for the purpose of replacing chlorine as the sanitising agent in the produce washing system. These included, (i) MCV-resin system (iodine), (ii) Mixed oxidant system (HOCl, ClO_2, O_3), (iii) Tsunami™ (peracetic acid) and a (iv) Silver / hydrogen peroxide system.

Shredded lettuce, carrot battons and broccoli florets were immersed in a washing bath via a conveyor system, then spin dried and packaged for storage and testing. Microbiological counts were performed before and after washing and following storage (shelf-life testing). In addition, sanitiser levels were monitored, microbiological counts performed on the washing water and ATP-bioluminesence to provide an indicator of the build up of organic levels in the washing water. These criteria provided an assessment of how effectively each sanitiser performed with increasing product throughput. Several factors that influence washing performance were investigated, including sanitiser performance at chilled water temperatures, pH and contact time. Also of particular concern to industry is the potential for pathogenic bacteria to survive the washing process. This was investigated by challenge trials with *Listeria monocytogenes, Salmonella spp.*, and *Escherichia coli* artificially innoculated onto shredded lettuce prior to washing with the respective sanitisers.

The findings for the pilot-scale washing trials and pathogen challenge testing are contained in the following reports submitted to the CRC / HRDC: (i) *Pilot-scale washing trial of minimally processed vegetables using MCV-resin technology*, December 1997; (ii) *Pilot-scale washing trial of minimally processed vegetables using Tsunami™*, April 1998; (iii) *Pilot-scale washing trial of minimally processed vegetables using a mixed oxidant system*, June 1998. (iv) *Pilot-scale washing trial of minimally processed vegetables using a silver / hydrogen peroxide system*, June 1998.

This report was designed to provide a cost-benefit analysis of the chlorine alternatives based upon: (i) microbiological criteria, (ii) operating and capital cost, (iii) ease of integration into existing washing practices, (iv) monitoring of sanitiser levels for a food safety program, (v) registration (ANZFA) and residue issues.

2. Objectives

To perform a cost-benefit analysis of (i) MCV-resin system (iodine), (ii) Mixed oxidant system (HOCl, ClO_2, O_3), (iii) Tsunami™ (peracetic acid) and (iv) a Silver / hydrogen peroxide system for the purpose of replacing chlorine as the sanitising agent to wash minimally processed vegetables

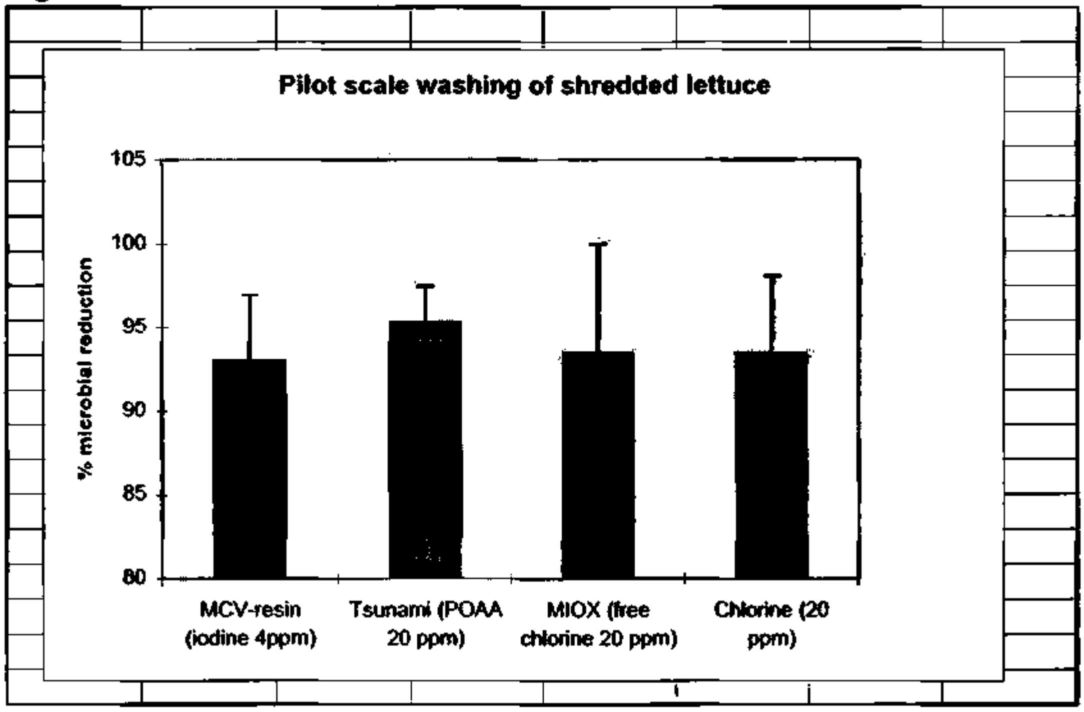
3. Performance evaluation of chlorine alternatives for the sanitation of shredded lettuce

3.1 Pilot-scale washing trials

The microbial reduction achieved on shredded lettuce using the MCV-resin system (iodine), Mixed oxidant system (HOCl, ClO_2, O_3), and (iii) Tsunami™ (peracetic acid) was similar to chlorine washing for the specified concentrations (Figure 1).

These treatments were not significantly different from chlorine, however the highest average microbial reduction was achieved using Tsunami™ which also had the smallest standard deviation. On this basis the most preferred chlorine alternative is Tsunami™.

Figure 1.



[Results sourced from a 30 kg shredded lettuce batch washing trials in 60 liters immersed for 3 minutes, from which product was sampled at 5 kg intervals (n=12)].

The silver / hydrogen peroxide system performed unsatisfactorily achieving only an average of 42% microbial reduction, which is well below an arbitary minimum requirement of 90% microbial reduction (or 1 log unit) to be marginally feasible.

The findings for the MCV-resin system are based upon the release of about 4 ppm of iodine into the water stream. Using water at a temperature of 12°C which is subsequently cooled before entering the washing bath. The level of iodine released can be increased by temporarliy raising the water temperature prior to passing through the MCV-resin. Trials performed up to 40°C, demonstrated that 7.0 ppm of iodine can be released. However this would incur the extra expense required for heat exchange.

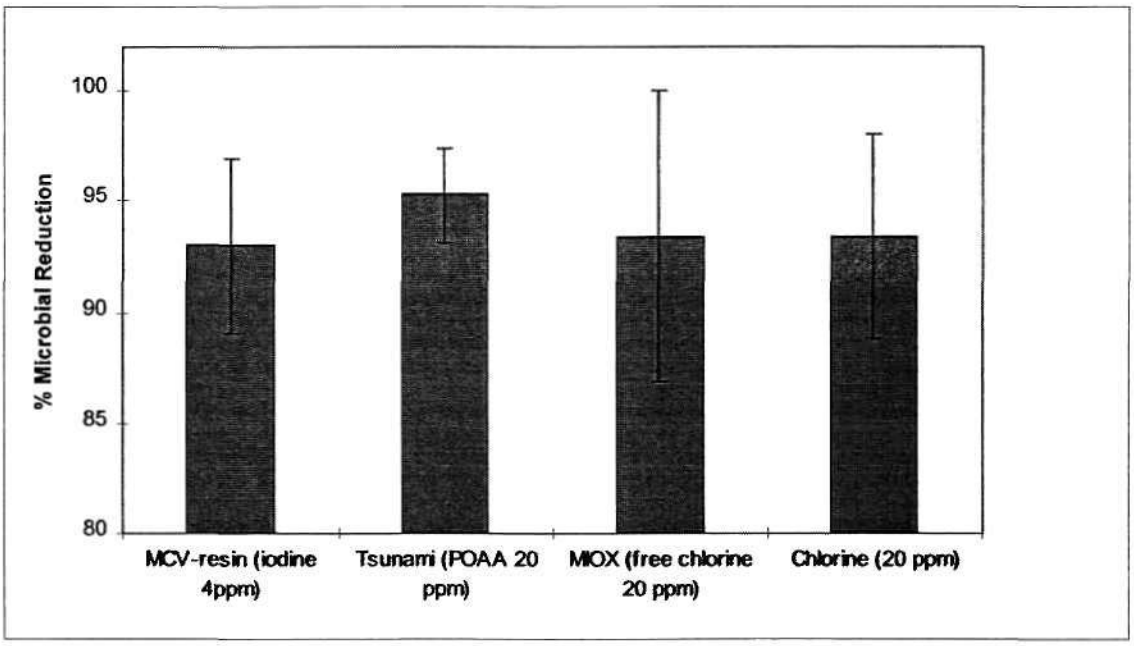
3.2 Pathogen challenge trials with Listeria monocytogenes, Salmonella spp., and Escherichia coli

For the different pathogen challenge trials the performance of the sanitisers varied considerably, except for Tsunami™. Overall, Tsunami™ at 50 ppm POAA was the most effective of the sanitisers consistantly providing a microbial reduction >90% for all three of the pathogens tested. At 10 ppm POAA, Tsunami™ did not meet this criteria.

Listeria monocytogens

Tsunami™ was the only product that provided greater then 90% reduction in *Listeria monocytogens* population, achieving 97.3% and 98% reduction at pearacetic acid levels of 10 ppm and 50 ppm respectively (figure 2). This performance could not even be matched using mixed oxidant water treatment at a free chlorine equivalent of 60 and 120 ppm. The results for silver / hydrogen peroxide treatment are not shown because a minimal reduction of 48 %was achieved.

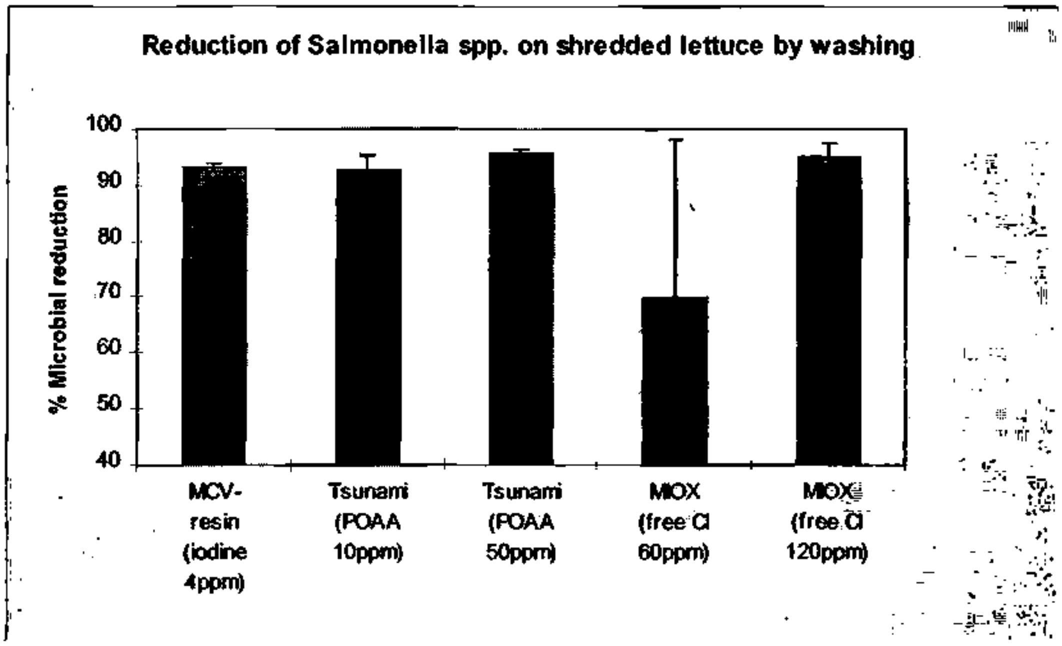
Figure 2. Pilot-scale washing of shredded lettuce



Salmonella spp

A similar reduction in the Salmonella populations was achieved using MCV-resin (iodine 4ppm), Tsunami (POAA 50 ppm) and mixed oxidants at a free chlorine equivalent of 120 ppm (Figure 3). No three sanitising systems could not be seperated upon sanitation performance. The results for silver / hydrogen peroxide treatment are not shown because a minimal reduction of 28 %was achieved.

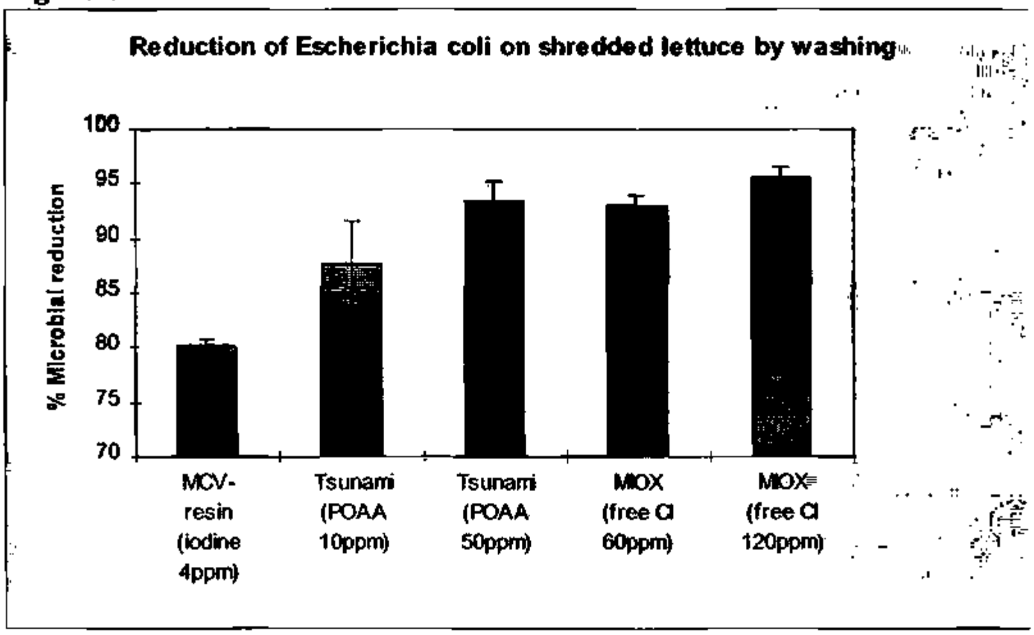
Figure 3.



Escherichia coli

The MCV-resin system (iodine 4ppm) and Tsunami at a POAA of 10 ppm did not provide a satisfatory reduction in the level of *E.coli* (ie > 90%). A satisfactory reduction was achieved using higher levels of peracetic acid (POAA = 50ppm) and even higher levels of free chlorine equivalent for the MIOX system (Figure 4).

Figure 4



3.3 Performance of sanitisers when increasing organic load in the washing system

During the Tsunami batch washing trial the peracetic acid levels declined by 50% (from 20 ppm to 10 ppm), at a very similar rate to free chlorine levels during the chlorine batch washing trial. At the reduced levels both Tsunami and chlorine provided acceptable microbial product sanitation (ie >90%). These trials indicated that the performance of Tsunami was comparable with chlorine washing.

The MCV-resin batch washing trials resulted in the rapid depletion of iodine to ineffective levels, following the washing of only half the product washed in the chlorine and Tsunami trials. However, when the water was recycled through the resin the iodine level in the washing bath was maintained at an effective level (ie. > 3ppm). When used in a continuous recycling system filtration is required to reduce the organic load in the recycled water, otherwise the iodine will be partially depleted prior to entering the washing bath.

The mixed oxidant system predominantly consists of hypochlorous acid hence this species is depleted at the same rate as chlorine. In addition the ozone is known to be rapidly depleted because it is unstable.

4. Operating and capital cost

The MIOX and silver systems each require a substantial capitial expenditure for the sanitiser generating equipment, also the operating costs for such systems are not cost competitive when compared with chlorine. In addition these systems may also require maintenance (ie. electrode replacement) and have a limited operational life-time, this will incur additional costs not factored into the assessment presented in Table 1.

The Tsunami and MCV-resin iodine are more cost effective as their is no substantial captial expenditure requirement for a system to generate the sanitiser. The MCV-resin system requires purchase of the resin and the cannister to situate the resin online. The MCV-resin requires requires regeneration of theiodine, for which an estimate is included in the operating costs (Table 1). These two systems would also suit small processors because there is little or no captial expenditure requirement.

The operating costs of MCV-resin and Tsunami are comparable to chlorine washing. The MCV resin system is expected to require iodine regeneration after treatment of about 100,000 litres. However this will depend on the quality of the water and wether or not wash water recycling is undertaken. The operating cost of MCV-resin and Tsunami could be offset by providing further cost benefits compared with chlorine in the way of (i) reduced equipment corrosion, (ii) less gassing off, (iii) improved sanitiser stability, and (iv) reduced costs associated with the disposal of chlorinated water to meet E.P.A. requirements. However, these cost reduction benefits need to be verified by a commercial trial.

Table 1. Summary of cost requirements for each washing system

	Chlorine (50 ppm)	**Tsunami (50 ppm)**	**MCV-resin Iodine (4 ppm)**	**MIOX (50 ppm)**	**Ag^+/H_2O_2 (0.1 ppm/0.04%)**
Capital cost	None	None	Resin, cannister, $ 600	electrolytic cell, $ 15,000	electrode system $ 30,000
Operating cost*	$ 83	$ 150	Recharge resin $ 90	salt, power $ 300 (salt)	H_2O_2 , power $ 300
Total Cost	**$ 83**	**$ 150**	**$ 690**	**$ 15,300**	**$30,300**

* 100,000 Litres of treated water

Note: Includes cost estimates from chemical and equipment suppliers of product and services some of which are not yet marketed in Australia. The costings will vary upon the quality of the water and washing system design.

5. Integration of chlorine alternatives into existing washing systems

5.1 Washing system design and application

The **Tsunami™** product was very convenient to use, being applied in the same manner as chlorine from a concentrated stock solution. The washing bath can be dosed manually or by a pump system that is controlled via milli-volt readings from an oxidative redox potential (ORP) probe. The later method is designed to maintain POAA levels in continuous water recycling washing systems and reduce worker exposure.

The **MCV resin** system is fitted in the same manner as as filter cannister on-line to the water supply used to fill a washing tank or situated in the recycling water line. Water should not contain residual chlorine. We used town water which had been chlorinated and found that this did not cause a problem, but if the chlorine levels in the water source are higher, this can result in an increase of the iodine released from the resin. The system is very convenient to use, avoiding the requirement to handle chemical concentrates. Different sized resin cannisters can be purchased to accomodate larger flow rates. However, the release of iodine is reduced when using chilled water and this can be overcome in a batch operation by iodinating the water prior to chilling, or in a recycling system using a heat exchanger in-line to warm the water prior to passing through the resin and then recooling before re-entery of the solution to the water bath.

The **mixed oxidant system** is designed to continually treat a stream of water with hypochlorous acid, chlorine dioxide and ozone. The system produces the oxidants at approximately operational concentration so that the washing bath can be filled directly. The flow rate is limited by the size of the electrolytic cell. Water can be treated prior to washing but stored for only a short period as the oxidant will gas-off. For a continuous washing recycling system the sanitiser dosing will require water replacement to maintain the oxidant levels, thereby using considerably more water than that used in applying a sanitiser concentrate (ie. chlorine and Tsunami). Also accuracy in maintaining oxidant levels would be very difficult. Overall the system is not very practical for the washing of fresh-cut vegetables.

The ***silver / hydrogen peroxide system*** system is of similar design to the MIOX system in that the water passes through an electrolytic cell and the treated water produced is at operational concentrations. Therefore it has the same functional problems associated with the MIOX system. In addition to this process/online monitoring of the silver levels is not possible particularly at low levels. Therefore this system is not a practical alternative for the washing of fresh-cut vegetables.

5.2 The influence of product sanitation contact time requirements on washing system design.

Contact time trials were performed to determine the suitability of using each sanitiser as a spray or for the shorter immersion periods of fast moving flumes or for longer immersion periods of larger washing tanks. The washing time parameters included spraying (equivalent to about 5 seconds), immersion for 30 seconds, 1 minute and 3 minutes. The performance of each sanitiser was based upon microbiological critieria for each washing time.

Tsunami (peracetic acid) was the only sanitiser tested that was very effective for both spraying and all of the immersion times. Therefore this product could be applied to a wide variety of different washing system designs, including spray conveyors, flumes and washing tanks.

The mixed oxidant system was more effective when the product was immersed,indicating that it was less suitable for spraying and more applicable for flumes or washing tanks.

Both the MCV-resin iodine system and the silver/hydrogen peroxide systems provided most effective sanitation when the product was immersed for an extended period of 1 to 3 minutes. Indicating that these systems are more applicable for washing tanks.

6. Monitoring and dosing of sanitiser levels

The main active ingredient for Tsunami™ is peracetic acid (POAA), which can be monitored rapidly on the spot with a colour indicator dip strip (supplied by Merck), or an Oxidative redox potential (ORP) probe is recommended by Ecolab (US) to continually control the dosing of a water stream for continuous washing systems. Titrations can be performed to determine the POAA levels, however this technique requires specialist training and expertise and there is an unsatisfactory time delay which does not suit processing operations. The peracetic acid test strips cost about 65 cents per test.

ORP and pH controller system with peristaltic pumps ranges from $3000 to $7000. This system should also include a chart recorder digital logging of sanitiser levels for monitoring records, adjustable settings and an alarm when concentrations fall outside a preset range.

Iodine levels can be monitored using DPD reagent and a calibrated colorimeter in the same manner as that for chlorine. A hand held colorimeter with digital display of sanitiser concentration cost about $300 to $350 and about 30 cents a test for the reagents.

The silver levels can be monitored using an ion-selective electrode. However, this method has not yet been validated for a vegetable washing system. The silver levels during the pilot-scale trials were determined by atomic absorption spectrometry.

7. Registration and residue issues

Tsunami™

Tsunami™ breaks down to acetic acid, water and oxygen. With use of a test strip, applied to the surface of washed lettuce, no POAA (LOD=5ppm) could be detected after 1 hour, prior to microbial sampling. Therefore this product can be used without requiring a final water rinse, the current practice for chlorine operations. Currently peracetic acid is permitted as a washing agent specified by ANZFA, Standard, A16, group II. However this is a different form of peracetic acid and an application for registration has yet to be sought by Ecolab®, the manufacturer of the products.

Silver levels

Currently silver is permitted by ANZFA for use in drinking water at a maximum level of 0.1 ppm. Consultation with ANZFA suggests that if silver was to be permitted as a washing agent a maximum residue limit of 0.1 ppm would also apply to the washed produce. Hydrogen peroxide is permitted as a washing agents specified by ANZFA, Standard, A16, group II. Information to date indicates that the system produces more silver ions than 1 ppm and analysis of product residues are still to be perforemd by consulting laboratories, at the time of writing.

Iodine

Treatment of vegetables with iodine levels up to 75 ppm has resulted in minimal iodine uptake. A report prepared by for MCV Technologies (Stephen Morris from CSIRO, Sydney Postharvest Laboratory), suggested that any increase in the iodine level of horticultural produce would probably be less than that due to the natural differences arising from different soil types. The level of iodine in horticultural produce varies considerably depending on the iodine levels in the soil in which the produce was grown. Residue testing of shredded lettuce washed with iodinated water (4 ppm) failed to detect any iodine using a surface washing method at a detection level of 0.1μg/mL. supplies of th technology would be keen to pursue registration of the product as a washing agent.

MIOX

Testing results supplied by Containation Control Ltd indicate that the mixed-oxidant solution produces from 20 to 50 percent the total trihalomethanes (TTHMs) as does hypochlorite on an equal chlorine basis. This reduces the problems associated with by-products but is not as favourable as a chlorine free alternative without any other residual concerns. The compnents of the mixed oxidant system are all permitted washing agents specified by ANZFA, Standard, A16, group II.

8. Conclusions

Overall Tsunami was the most effective chlorine alternative based on microbiological. In addition the product can be introduced into washing systems in a similar fashion to chlorine, applicable for spraying, fast flumes and washing tanks. Tsunami was also the most cost effective of the chlorine alternatives investigated in this study.

9. Further work

*Assist industry implementation of chlorine alternative washing practices, via commercial trial with Tsunami

*Develop continuous monitoring system for larger scale operations using Tsunami

*Enhancement of washing system by the use of immersible ultrasonic transducers.

Milestone #7: Industry consultation: chlorine alternatives

EXECUTIVE SUMMARY

To date Lloyd Simons and John Coventry have conducted meetings with several of Australia's major fresh-cut processors. The purpose of the visits were to discuss the CRC/HRDC pilot-scale washing trials and facilitate the introduction of a chlorine alternative into the fresh-cut industry by the way of a commercial washing trial.

Several commercial requirements were identified, which included the following: (i) microbiological efficacy, (ii) reduced gas-off, (iii) stability of sanitisers, (iv) on-line monitoring, (v) environmental and health issues relating to by-products and residues. Most of these issues were targeted during the pilot-scale trials completed last year. However, pilot-scale processing is limited in the way it can reflect large volume production runs. The commercial trials will be designed to assess these issues.

Arrangements have been made to conduct a commercial washing trial with Keilor Valley Gardens in January. This will include, assessment of the existing chlorine washing system for iceberg and mesculin lettuce mix, then a comparison made using Tsunami. These trials will be performed in conjunction with Ecolab. The association with Ecolab has also facilitated trials at Harvest Fresh-cuts, which will be conducted in-house independently of this project. However acess to outcomes will be provided on a confidential basis.

INTRODUCTION

11. SUBPROJECT BACKGROUND:

During 1997-98 several pilot-scale washing trials were conducted at Food Science Australia to evaluate the performance of chlorine alternatives for the washing of fresh-cuts. Evaluations were based on i) microbiology of product and water, (ii) integration into existing systems, (iii) monitoring and dosing, (iv) cost and (v) registration (ANZFA).

The following reports were submitted to HRDC or CRC.

Pilot-scale washing trial of minimally processed vegetables using MCV-resin technology

(March, 1998); (2) *Pilot-scale washing trial of minimally processed vegetables using mixed oxidant system* (June, 1998); (3) *Pilot-scale washing trial of minimally processed vegetables using a silver / hydrogen peroxide system* (June, 1998); (4) *Pilot-scale washing trial of minimally processed vegetables using Tsunami* (June (1998); (5) *Cost benefit analysis for chlorine alternatives to sanitise minimally processed vegetables* (June, 1998).

Pilot-scale processing is limited in the way it can reflect large volume production runs with higher organic loading. A commercial trial is needed to conclusively examine sanitiser performance. Of the chlorine alternatives investigated, Tsunami was considered the most appropriate for proceeding with an industry trial.

PROJECT AIM

Consultation with key fresh-cut industry processors regarding the feasibility of trialing a chlorine alternative.

To identify an alternative to chlorine washing to sanitise fresh-cut vegetables based on pilot-scale processing trials, using microbiological criteria as a measure of sanitation performance. Then facilitate the commercialisation of an alternative washing system by assisting in trials at a commercial fresh-cut processing facility.

CONSULATATION PROCESS

Several visits have been conducted to leading Fresh-cut Processors in Victoria, New South Wales and Queensland.

These industry visits involved the following discussions:

(i) Outcomes from the CRC pilot-scale washing trials conducted at Food Science Australia.

(ii) Canvassing current industry issues in relation to chlorinated washing, and it's limitations.

(iii) Process improvement requirements for a chlorine alternative.

(iv) Facilitating the commercialisation of a chlorine alternative into the fresh-cut industry with an industry washing trial.

Summary of industry visits:

1. Harvest Fresh-Cuts (Qld)*
2. Mrs Crockets Kitchens (Qld)
3. All States (NSW) (now owned by Mrs Crockets Kitchens)
4. Moraitis (NSW)
5. Salad Fresh (Vic)
6. Keilor Valley Gardens (Vic)

* including Vegco (Vic) and Scalime-Food Processing Technologies (France)

OUTCOMES OF INDUSTRY CONSULTATION

Industry issues for chlorine washing and process improvement

Several requirements for a chlorine-alternative were identified by industry representatives. These were usually based upon commercial limitations currently encountered with chlorine washing, including the following:

(i) **Microbiological efficacy:** Verification of the sanitation performance based upon bacterial counts of the product before and after washing and during shelf-life storage. This would enable processors to make a comparison with existing microbiological performance data for chlorine washed products.

(ii) **Gas-off**: Currently washing with chlorinated (and chlorine dioxide) water presents occupational health and safety (OH&S) issues. Even at levels below the threshold limit values (TLV's) some workers can become sensitised. This is especially apparent in washing systems that incorporate air-jets and are situated in enclosed areas for hygiene requirements. Therefore a chlorine-alternative that is less toxic and /or with a lesser tendency to gas-off would be more favourable.

(iii) **Stability of sanitisers:** Preference for an alternative that has more stability than chlorine under high organic loads. This is particularly important for products which are known to release high levels of organic material that rapidly reduce chlorine (ie. finely shredded lettuce, capsicum slices).

(iv) **Monitoring:** Requirement for an easy and rapid monitoring test for sanitiser levels. Large scale processors require continuous on-line monitoring and dosing control.

(v) **Environmental and health issues relating to by-products and residues:** Preference for an alternative that does not have the problem associated with chlorinated by-products.

(vi) **Registration:** Requirement that a sanitiser can be listed as a washing agent within the Australian and New Zealand Food Standard Codes.

(vii) **Cost:** Identification of any cost savings which may be attributable to improved stability or solubility and reduction in OH&S problems. The issue of cost for the larger processors is reduced by the fact that sanitiser costs represent a proportionally smaller component of the turnover. However in the case of smaller processors cost is a more important consideration.

Establishment of Industry trials for Tsunami

Arrangements have been made to conduct a commercial washing trial with Keilor Valley Gardens in January. This will include, assessment of the existing chlorine washing system for iceberg and mesculin lettuce mix, and a comparison made using Tsunami. These trials will be performed in conjunction with Ecolab who will be providing technical support for dosing and monitoring with Tsunami, based upon application experience in the United States. The assessment of the sanitiser will be performed to take into account issues raised during discussions with industry, in particular microbiological criteria, sanitiser stability under high organic loads and gas-off.

Keilor Valley Gardens has agreed to the publication of trial results subject to their approval. Trials will commence in January 1999 and be completed by March 1999.

Also trials at Harvest Fresh-cuts have been facilitated with support from Ecolab. These will be conducted at Harvest Fresh-cuts independently but access to the outcomes will be provided on a confidential basis.

Milestone #8: On-line sanitiser control for continuous monitoring and dosing

EXECUTIVE SUMMARY

For large-scale fresh-cut processing facilities that operate continuous recycling washing systems, on-line dosing is more suitable then a batch dosed operation. The washing system is a dynamic process that is continuously changing in product throughput and product types, which result in different sanitiser demand requirements. On-line monitoring can provide more accurate critical control point monitoring, operational control, and recording for HACCP-based food safety programs. Previous research had identified Tsunami as a favourable alternative to chlorine for fresh-cut washing operations based upon pilot scale washing trials (*Cost-benefit analysis for chlorine alternatives to sanitise fresh-cut vegetables,* for CRC-International Food Manufacture & Packaging Science, June 1998*)*. To facilitate industry introduction, pilot-scale on-line monitoring and dosing trials were conducted. Initially, dose response studies were performed to correlate oxidant value (mV) with the peracetic acid concentration in the washing water and an operating range and limit settings were established. The ORP system for monitoring peracetic acid was found to be effective for concentrations up to 10-20 ppm. Pilot-scale trial of a continuous water recycling washing system was successfully conducted with shredded lettuce. Further trials are required to evaluate the redox on-line monitoring and dosing for a Tsunami system under commercial conditions

INTRODUCTION

Pilot-scale washing trials were conducted at Food Science Australia and a confidential project report was submitted to the CRC-International Food Manufacture and Packaging Science, June 1998. These trials demonstrated the suitability of Tsunami for washing minimally processed vegetables based on microbiological criteria. The washing process is a dynamic system where the level of free available active sanitiser changes due to increasing levels of organic material, temperature, pH and continual gassing-off (depends on washing system design). Monitoring of the sanitiser level is a critical control point for the production of minimally processed vegetables, ensuring that specified effective levels of the sanitiser are maintained throughout the processing operation. During the pilot-scale trials monitoring of the peracetic acid levels was performed using test strips for the washing water and titration of the concentrate stock solution. The test strip method is convenient and cost effective for small processors, particularly those performing batch-washing operations. However, for processors that use water recycling systems or flow through systems for large quantities of product, an on-line monitoring and dosing control is more favourable, provided monitoring and dosing can be performed accurately to maintain sanitiser levels within a satisfactory operating range. Currently, a few large processors use an oxidative redox potential (ORP) system for the on-line monitoring and dosing control of chlorine. One of the key criteria for the commercial application of an alternative to chlorine for washing minimally processed vegetables is the requirement for a commercially available and practical on-line monitoring and dosing system.

PROJECT AIM

Evaluate the operational effectiveness of an oxidative redox potential on-line monitoring and dosing system for Tsunami™ (peracetic acid) in a pilot-scale washing system.

METHODS

On-line monitoring and dosing system

A HP2AS dosing system package was purchased from Crown scientific, consisting of a controller for monitoring and dosing sanitiser and pH, 2 peristaltic pumps (3L/Hr), pH probe, oxidative redox potential probe and temperature compensation probe.

Preliminary bench-scale calibration of oxidative redox potential (ORP) with peracetic acid concentration

The pH probe, ORP probe and temperature compensation probe were positioned in a flow through chamber and submerged in a 20 litre tank of water. The HP2AS system was set up to monitor both the ORP and pH, while the peracetic acid concentration was monitored using peracetic acid test strips (Merck). Peracetic acid was progressively added to the tank.

Pilot-scale washing system with continuous on-line sanitiser dosing and monitoring.

Initial set up and operation was established for redox and pH probes, sanitiser and acid dosing points to ensure, adequate mixing of the washing agents, to minimise over-dosing and under-dosing, and maintain peracetic acid levels in the washing tank .

Summary of operating parameters;
Peracetic acid 50 ppm.
Flow rate of recycling water 700 L/hour.
Product immersion time of 3 mins (conveyor speed setting of 0.5).
Continual operation of a spray fan jet (200/hour) and 5 air jets.
Volume of washing bath 60 litres.
Total volume of recycled water including overfill tank 120 litres.
Filters included 2 mm screen, 150 μm basket filter and 20 μm filter.
Product load rate of about 5 Kg / 6 mins.
Temperature of water in the washing bath 3.6°C

A total of 30 Kg of shredded lettuce (1 cm width) was washed. Testing was performed at 10 Kg intervals for the following; microbiological testing of product and water, measurement of organic load in the water using ATP-bioluminescence, ORP and pH, peracetic acid concentration using peracetic acid test strips (Merck).

Microbiological and physiological shelf-life testing.

After washing, the shredded lettuce was spin-dried and packaged in poly-propylene RC004 (Cryovac) with an OTR of about 4,000, and stored at 4°C. Products were sampled in triplicate for microbiological analysis. A total of 20 gram portions was homogenised (Colworth stomacher) in 180 mL of 0.1% bacteriological peptone (Oxoid), then spiral plated on plate count agar (Oxoid) and incubated for 2 days at 30°C.

Packaged headspace gas analysis of carbon dioxide and oxygen content was analysed using MAP test 400 equipped with an infra-red carbon dioxide analyser and a zarconia sensor for oxygen.

Visual assessment was performed to identify any physiological disorders (ie. browning, mid-rib pinking, physical damage etc.).

RESULTS

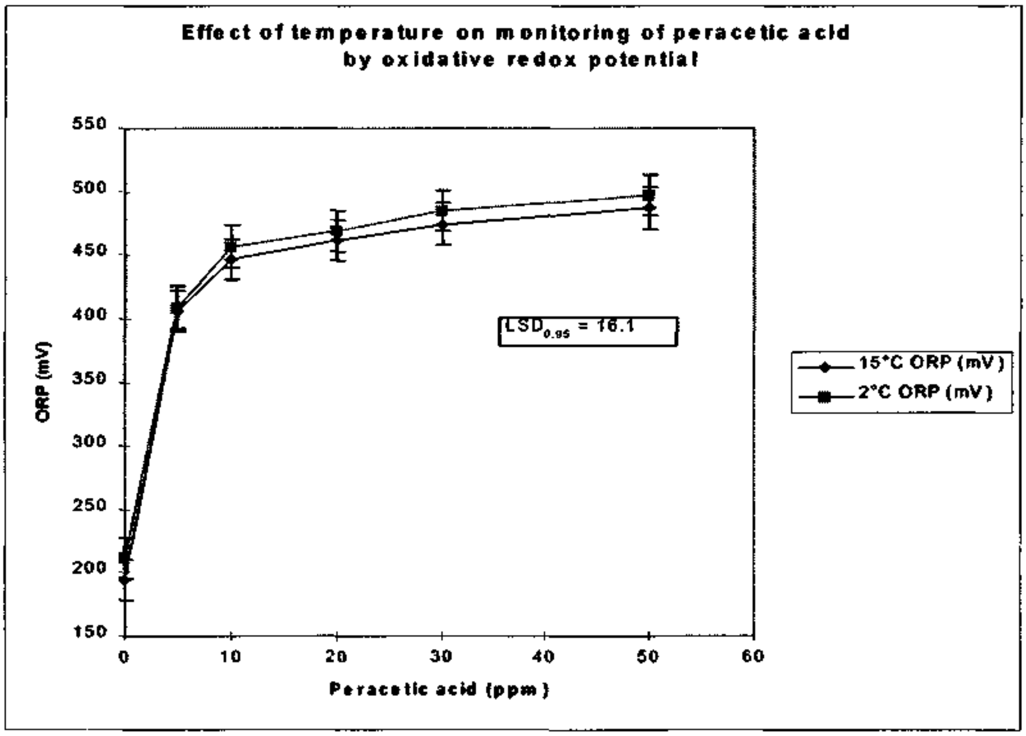
Preliminary bench-scale calibration of oxidative redox potential with peracetic acid concentration

The ORP (mV) in untreated water was approximately 200 mV. The cumulative addition of Tsunami to the water showed that the ORP (mV) readings increased rapidly to approximately 400 mV with the addition of 5 ppm of peracetic acid (Tsunami). With subsequent additions of Tsunami, the ORP mV readings rose at a reduced rate with increasing levels of peracetic acid. Consequently, the accuracy of controlling the peracetic acid levels is reduced at higher levels. There was a significant difference ($p=0.05$) in ORP mV readings between peracetic acid levels of 5, 10, 20, 30 and 50 ppm, which is considered the operational range. The pH was not adjusted during the trial, consequently it declined with increasing levels of peracetic acid (see Figure 1).

The washing of fresh-cut vegetables is generally performed using chilled water, therefore the Tsunami dose and ORP response was performed at 2°C, to determine if the temperature compensation probe was operating appropriately (see Figure 2).

Figure 1.

Figure 2.



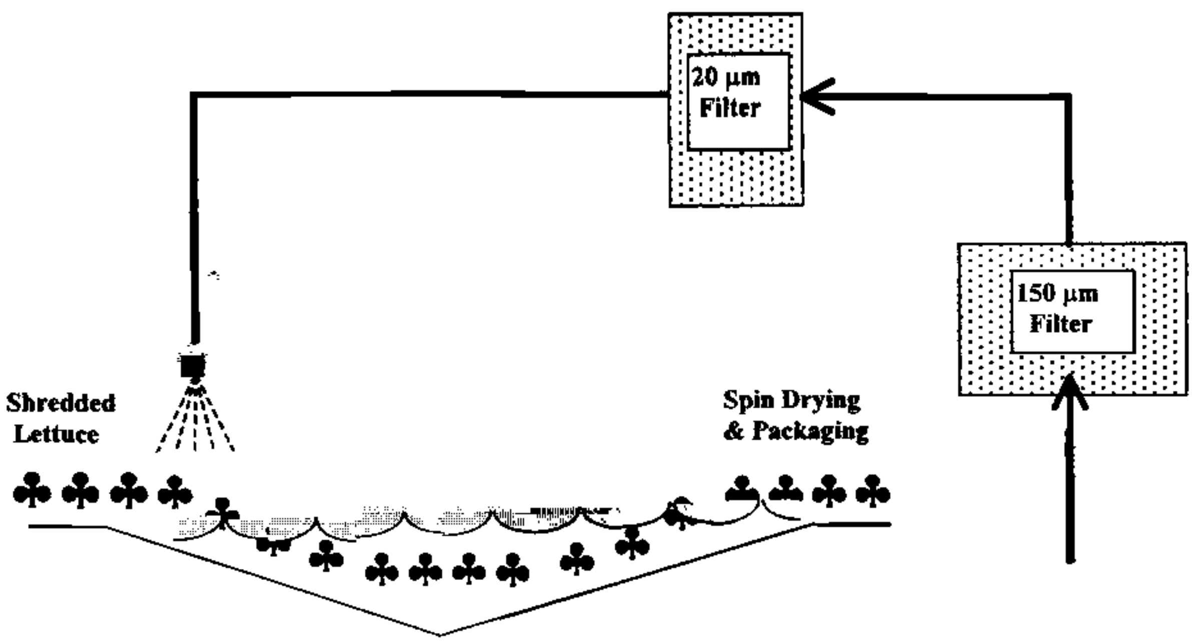
The results indicated that if the temperature of the washing water changes considerably during processing (2°C to 15°C) the ORP calibration curve could drift sufficiently to alter the dosing control. 20 ppm the ORP system it could not differentiate it from 30 ppm. At 2°C the ORP system did not provide a significant difference ($p=0.05$) between 10 and 20 ppm or higher dosing intervals of peracetic acid. When at 15°C the ORP system ($p=0.05$) did not provide a significant difference between 20 and 30 ppm of peracetic acid. At lower levels of peracetic acid the ORP system was effective in differentiating between dose intervals. Further testing would be required to evaluate the system at a range of temperatures to accurately quantify the drift in ORP readings. During processing the water temperature can be maintained with the use of a heat exchanger to minimise this effect.

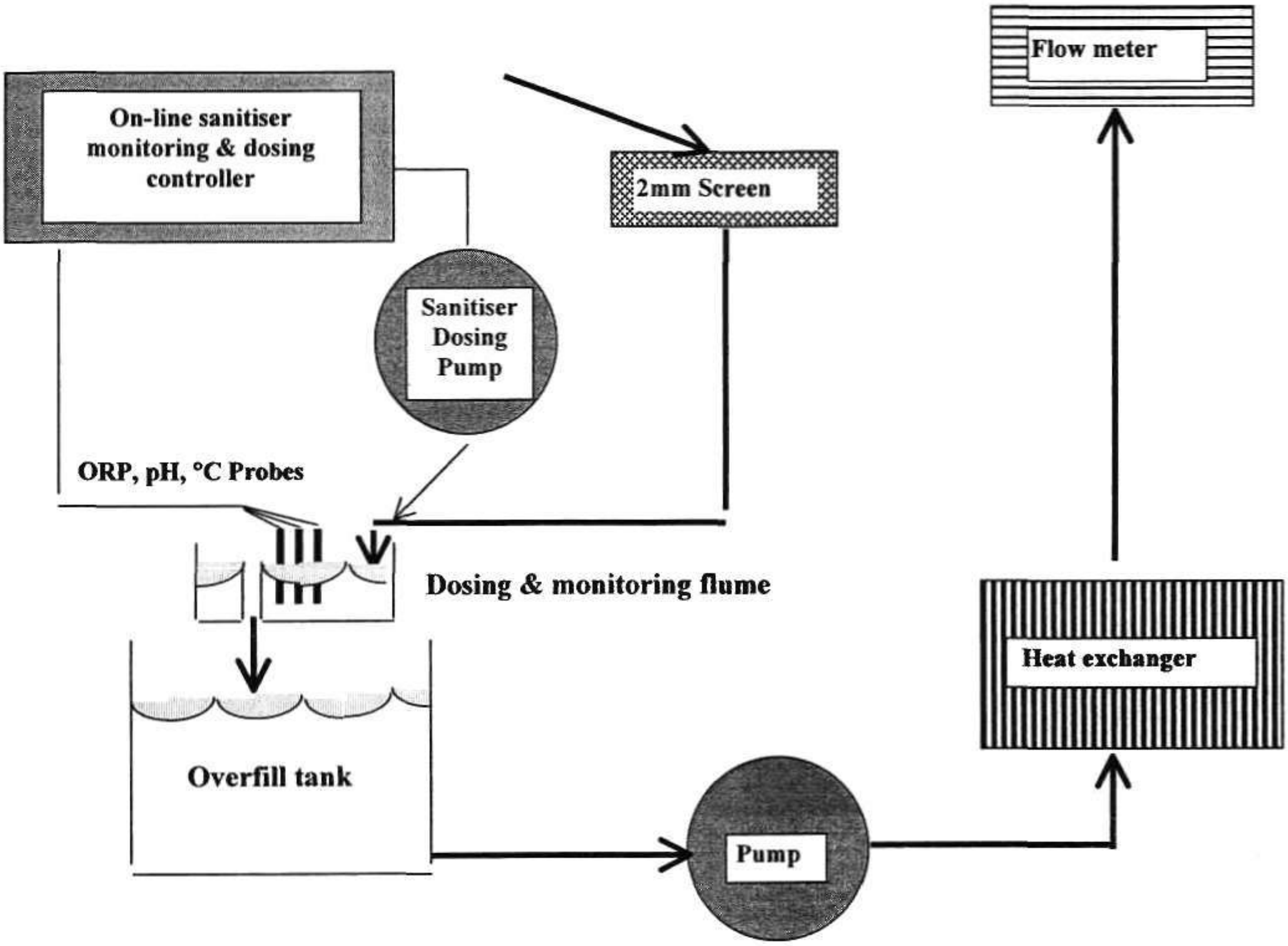
In laboratory trials the water is very clean, whereas during commercial operations there are high organic loads which may provide further interference with the ORP mV readings. It is therefore important that the redox on-line monitoring and dosing control is validated under commercial conditions. Further variation will be encountered for the amount and type of organic matter released by different products. The ORP controller must be calibrated for a particular sanitation system and regularly validated using conventional testing for peracetic acid (ie. test strips) or titrations.

Set-up of factory pilot-scale washing system:

The water in the washing system was recycled and replaced every 10-12 minutes. Recycled water was dosed on demand to maintain a pre-set ORP minimum limit. Over-feed was minimised by situating the dosing line close (10cm upstream) to the ORP probe. To ensure proper mixing of the sanitiser the monitoring was conducted in a small flow-through mixing flume situated prior to the overfill tank. Another alternative to this would be to situate the dosing point and probes into a closed flume. For this washing system the monitoring probes could not be situated in the washing bath due to a double conveyor system. However, if the probes were to be placed directly into the washing bath there would have to be enough circulation to ensure even mixing and further measures taken to protect the probes from fouling with vegetable material. Situating the monitoring probes on-line in the recycled water stream was more practical, providing the flow rate is sufficient to replenish the washing bath with freshly dosed water at a sufficient rate to meet the product demand (see Figure 3).

Figure 3. On-line sanitiser dosing and monitoring washing system





Pilot-scale washing system with continuous on-line sanitiser dosing and monitoring.

Throughout the shredded lettuce washing trial the level of peracetic acid was maintained at 50 ppm, using the on-line redox controller (see Table 1). In comparison with declining peracetic acid levels observed in a batch washing trail which used the same weight of product, but without water recycling and a single dose at the commencement of processing (Milestone report on: *Pilot-scale washing trial of minimally processed vegetables using Tsunami*, June 1998). The organic load in the washing system increased during production as determined by ATP-bioluminescence readings (see Table 2). The accumulation of organic material was reduced by screening and filtration (to 20 microns) of the recycled water. The total mesophillic counts in the water remained low. A microbial reduction of 97.1%, 94.4% and 97.6% was achieved during washing at each of the testing intervals (see Table 3). The final microbial counts on the packaged product after washing was about 10^4 or slightly less, which from our experience is a commercially acceptable result. The shredded lettuce remained in a very good condition during the shelf-life trial (4°C for up to 11 days). There was some pinking of the vessels in the lettuce mid-rib and a very low incidence of browning, detected on only 1 or 2 leaves in a package. At 11 days the product had a minor loss in crispness. The package headspace atmosphere had reduced oxygen and increased carbon dioxide levels in a range that would reduce enzymatic browning (see Table 5). Considering that the product will probably be consumed by 7 days the storage quality was acceptable at 4°C.

In commercial operations with large production runs the organic loading in the water is considerably higher then that encountered in a pilot-scale washing system. Consequently there is an increased demand on the sanitisers, which is a more challenging operational test for the on-line redox monitoring and dosing system. Therefore, to appropriately test this system further trials will need to be conducted in a commercial operation.

Table 1. On-line monitoring and dosing control of Tsunami for washing shredded lettuce

Shredded Lettuce Cumulative total (kg)	Oxidant Redox (mV)	pH	Peracetic acid using test strip (ppm)
0	505	2.92	50
10	504	2.96	50
20	503	3.00	50
30	503	3.07	50

*** Redox set limit of 425 mV;** But overdosed leading to 500

Table 2. Microbiological count in washing water and ATP water quality indicator.

Washed Shredded Lettuce Cumulative total (kg)	Total Mesophillic Count in water (cfu/100mL)	ATP Bioluminescence (RLU)*
0	3	3
10	15	182,545
20	10	272,170
30	18	over load

*RLU = relative light units. Average of duplicate readings

Table 3. Microbiological quality of shredded lettuce from continuous Tsunami washing trial

Shredded lettuce treatment	Mean* (Log10cf/g)	% Reduction in cfu/g
Unwashed (control)	5.22 ± 0.33	—
10 kg washed lettuce	3.74 ± 0.24	97.1 %
20 kg washed lettuce	4.03 ± 0.17	94.4%
30 kg washed lettuce	3.51 ± 0.22	97.6 %

Table 4. Microbiological shelf-life storage trial of shredded lettuce washed in Tsunami (50ppm, refer table 1,2,3)

Days of storage at 4°C	Mean (Log10cfu/g)
0	3.84 ± 0.19
4	5.75 ± 0.70
6	6.27 ± 0.66
8	6.75 ± 0.42
11	6.55 ± 0.61

Table 5. Packaged atmosphere of shredded lettuce during shelf-life storage trial

Days of storage at 4°C	Package Atmosphere	
	% Oxygen	%Carbon Dioxide
0	21.1 ± 0.1	0.0 ± 0.0
4	5.58 ± 1.24	11.7 ± 0.9
6	1.65 ± 1.46	15.2 ± 2.0
8	0.42 ± 0.82	18.9 ± 2.3
11	0.01 ± 0.00	23.6 ± 2.6

DISCUSSION

The ORP system for monitoring peracetic acid was found to be effective for concetrations up to 10-20 ppm with a pilot-scale system. There was increased variation in the ORP readings when using washing water at different temperatures. Further testing would be required to evaluate the system at a range of temperatures to accurately quantify the drift in ORP readings. During processing the water temperature can be maintained with the use of a heat exchanger to minimise this effect. In laboratory trials the water is very clean, whereas during commercial operations there are high organic loads which may provide further interference with the ORP mV readings. It is therefore important that the redox on-line monitoring and dosing control is validated under commercial conditions. Further variation will be encountered for the amount and type of organic matter released by different products. The ORP controller must be calibrated for a particular sanitation system and regularly validated using conventional testing for peracetic acid (ie. test strips) or titrations.

FURTHER WORK

In commercial operations with large production runs the organic loading in the water is considerably higher then that encountered in a pilot-scale washing system.

Consequently, there is an increased demand on the sanitisers, which is a more challenging operational test for the on-line redox monitoring and dosing system. To appropriately test this system further trials will need to be conducted in a commercial operation

Milestone #8: Evaluation of chlorine and Tsunami washing systems for lettuce salads at a commercial processing facility

EXECUTIVE SUMMARY

The washing trials were designed to provide a comparison between current chlorine washing practices and Tsunami as a replacement for chlorine in a commercial processing facility. Performance of the sanitisers was based upon microbiological quality of the treated water and product, and stability of the sanitisers during high organic loading. Assessment was performed on a vacuum-packaged shredded lettuce product for food service and mesculin lettuce salad mix packaged in a lined box. Tsunami was more stable then chlorine when there was a high organic and soil load in the washing water. The microbiological reduction achieved by both sanitisers on the lettuce products was not significantly different. However, during storage the total mesophillic population grew faster on the Tsunami treated product attaining significantly higher counts then the chlorine washed product on days 6 and 9. The appearance of shredded iceberg lettuce following chlorine or Tsunami washing and storage at 4°C for 9 days was very good, without browning, but with a slight loss in crispness. The controlling of browning is also dependent on using permeable packaging to attain a modified atmosphere. A trace amount of hydrogen peroxide gas (<0.1 ppm) was detected directly above the washing tank, while no chlorine gas (<0.3 ppm) or acetic acid gas (<0.5 ppm) was detected. The washing system incorporated a large water jet manifold system with minimal aeration and without any occupational health and safety concern in relation to sanitiser and by-product gas-off.

INTRODUCTION

Based upon pilot-scale washing trials of several sanitisers at Food Science Australia (1997-'98), it was established that Tsunami™ (a wash water sanitiser with active ingredients of peracetic acid and hydrogen peroxide) was the most favourable for commercial applications. Discussions were undertaken with several of Australia's leading fresh-cut processes to identify a commercial processing facility at which to conduct washing trials. An agreement was arranged to conduct washing trials at Keilor Valley Gardens, in conjunction with Ecolab. The washing trials were designed to provide a comparison between current chlorine washing practices and replacement with Tsunami. Performance of the sanitisers was based upon microbiological quality of treated water and product, and stability of the sanitisers during high organic loading.

PROJECT AIM

To evaluate the suitability of Tsunami as a replacement for chlorine in a commercial processing facility.

METHODS

Description of washing system

Three stage washing system consisted of:

1. Sanitised washing tank of 3000 litres
2. Water rinsing flume #1
3. Water rinsing flume #2

Each washing stage has an individual water supply in a recycling tank. Following each washing stage is a de-watering conveyor. The washing water was chilled between 3-8°C.

Product description

Two lettuce products were evaluated:

1. Finely shredded iceberglettuce (3-5mm in width)
2. Mesculin lettuce salad mix with 9-13 lettuce types of baby leaves cut off at the stalk.

Organic load and turbidity of washing water quality

An indication of the organic load in the washing water was conducted by measuring ATP levels with a ATP-bioluminescence kit (Biotrace) and single-shot water dip sticks (Biotrace). This provided a measure of the vegetable juice and matter in the washing water.

Turbidity was measured by the absorbance of the washing water at 610 nm with a spectrophotometer (Varian, superscan 3), as a measure of the soil loading in the washing water.

Microbiological analysis

Products were sampled in triplicate for microbiological analysis, 20 gram portions were homogenised (Colworth Stomacher) in 180 mL of 0.1 % bacteriological peptone (Oxoid), then spiral plated in duplicate on plate count agar (Oxoid) and incubated for 2 days at 30°C.

Water samples were filtered through TTC filter pad (Sartorious), then placed on a moistened nutrient pad and incubated for 2 days at 30°C.

Package headspace gas analysis

Gas analysis of the packaged headspace for carbon dioxide and oxygen content. Packaged headspace was analysed using a MAPtest 4000 equipped with an infra-red carbon dioxide analyser and zarconia sensor for oxygen

Detection of gas released from sanitised washing bath

Drager tubes and a calibrated hand pump were used to sample gas from above the washing water (10-20 cm) for chlorine gas (5-80 ppm), acetic acid (0.3-5 ppm) and hydrogen peroxide (0.1-0.3 ppm).

Statistical Analysis

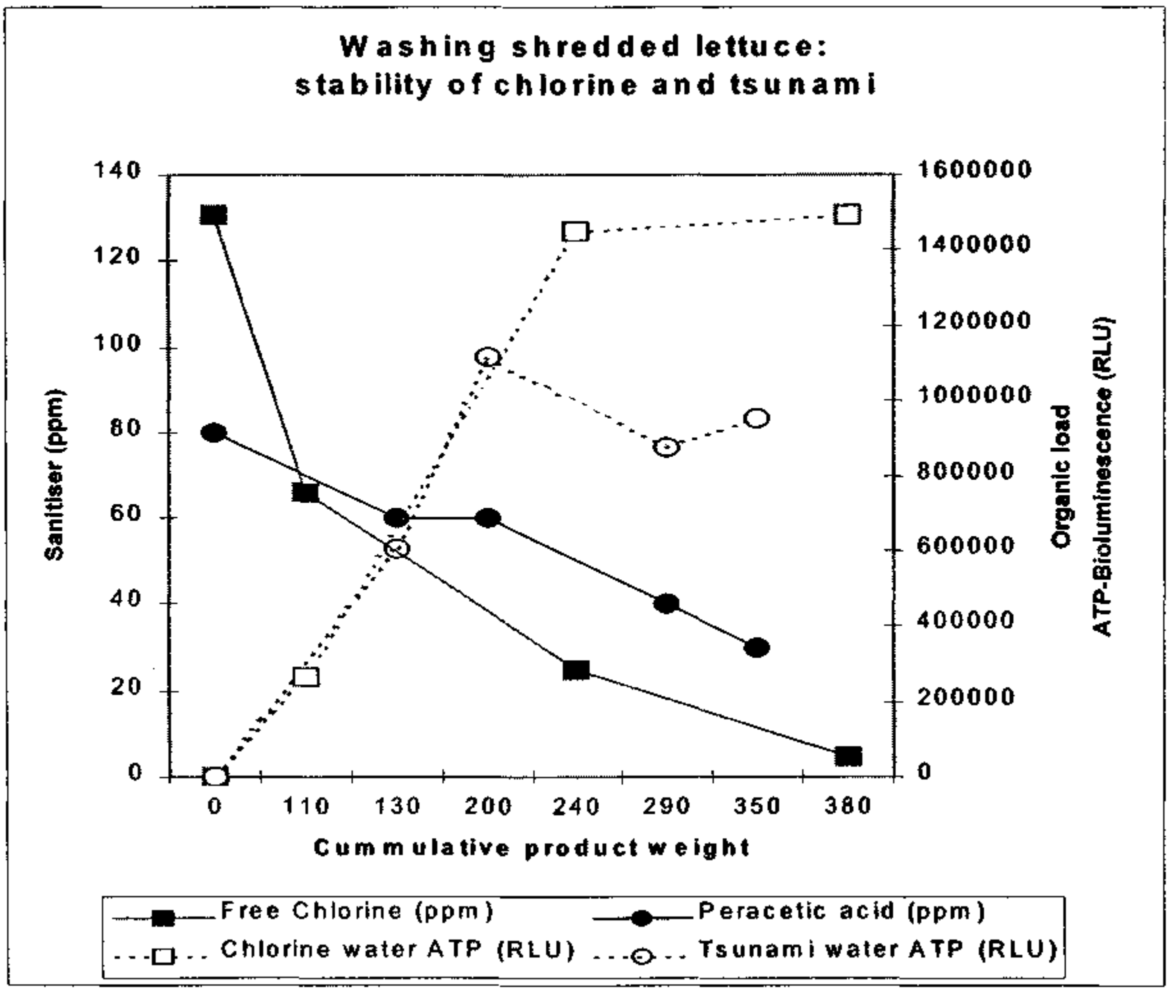
Mean of 3 replicate determinations are presented with respective standard deviation. Analysis of variance (single factor) was calculated using Genstat version 3.2 for calculation of least significant difference ($LSD_{0.95}$). Difference between mean values greater then $LSD_{0.95}$ is significant, at $p<0.05$.

RESULTS

Comparison of washing shredded lettuce with chlorine or Tsunami treated water:

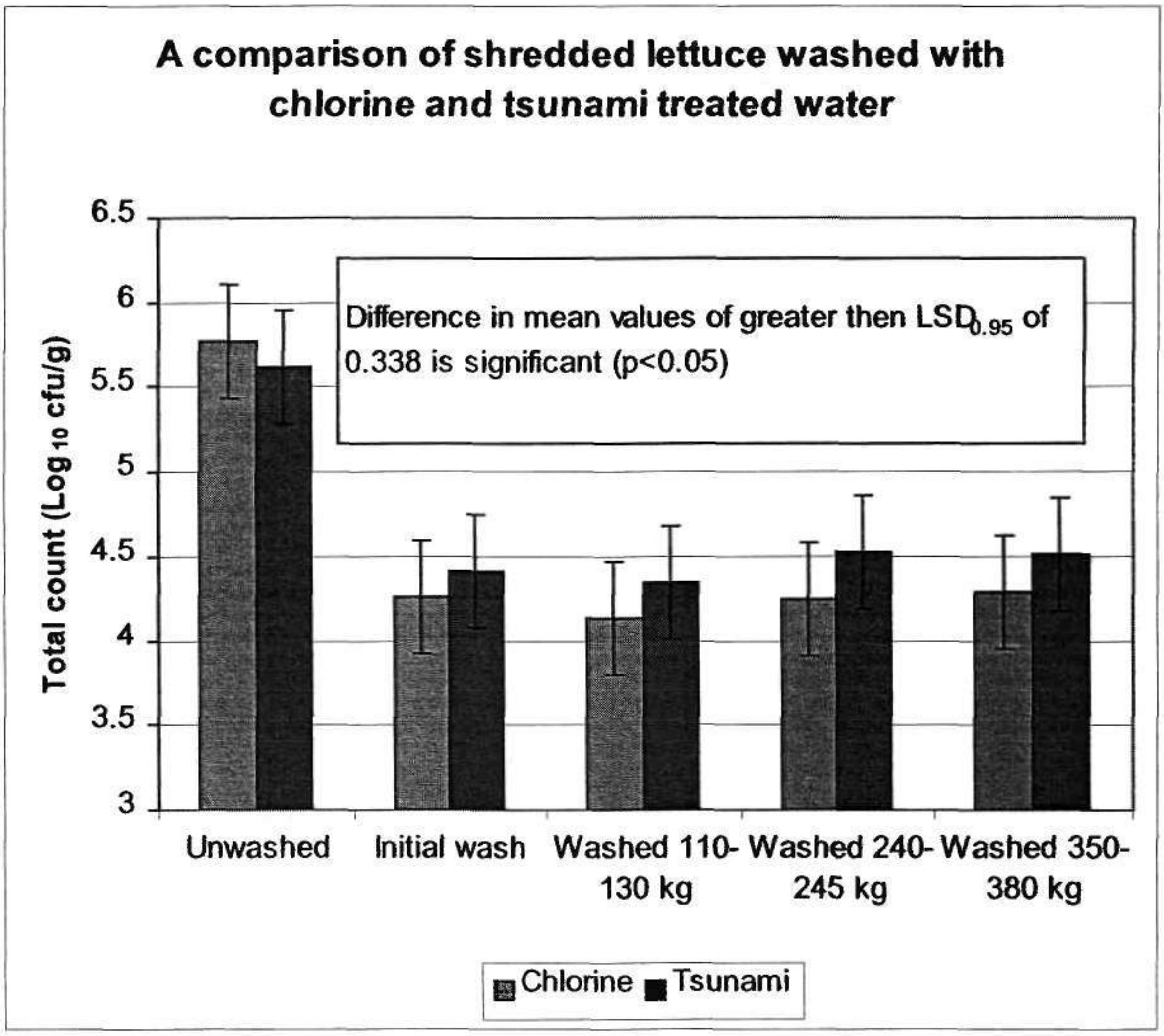
The initial level of free chlorine at 130 ppm rapidly declined throughout the processing shift to 5 ppm, following the production of 380 kg of shredded lettuce. Throughout this period the pH remained between 6.8-7.2. In comparison with Tsunami the peracetic acid commenced at 80 ppm and decline at a much slower rate to 35 ppm, following the production of 350 kg of shredded lettuce (refer Figure 1). This corresponded to a % decline of chlorine and Tsunami sanitiser of 3.8% and 38% respectively. During processing the organic load in both washing systems increased rapidly due to the release of organic material from the finely sliced shredded lettuce. Typically the washing system was operated with a semi-batch procedure involving filling the bath with shredded lettuce then circulating and holding for about 5 minutes before removal. However, for the Tsunami production a more continuous operation was performed in the later third of the processing shift, whereby the washing water was continuously recycled and screened. This resulted in the partial removal of the organic material as evident from the decline of the ATP levels after 200 kg of production (see Figure 1). By reducing the organic load in the washing system it should limit the usage of the sanitiser. However, taking this into consideration, the Tsunami is comparatively much more stable then chlorine under a high organic load. No chlorine gas was detected above the washing tank.

Figure 1:



Both the chlorine and Tsunami washing systems attained an average total mesophillic microbial reduction of at least 1 log. The microbial reduction was maintained throughout the processing shift (see Figure 2) despite the reduction in sanitiser levels (see Figure 1). The microbiological load on the raw product (unwashed) used for both the chlorine and tsunami washing not significantly different. Following washing in both chlorine and tsunami there was no significant difference ($p=0.05$) between the total mesophillic counts throughout the entire production shift (see Figure 2).

Figure 2:



There was a carry over of chlorine on the into the 1st and 2nd water rinsing flumes, attaining free chlorine levels of 16 ppm and 0.5 ppm respectively. The carry over of chlorine in small quantities was favourable for maintaining the microbiological quality (see Figure 3). The total mesophillic count in the 1st and 2nd water rinsing flumes remained low (see Table 1), despite the build up of organic material to an average of 479,470 RLU and 297,250 RLU respectively. There was also a carry over of Tsunami into the 1st and 2nd water rinsing flumes, attaining peracetic acid levels of 15 ppm and <5 ppm respectively (see Figure 3). The total mesophillic counts in the Tsunami treated washing water were considerably higher (1,120-3,560 cfu/100mL), then for the chlorinated washing system (see Table 1).

Table 1: Microbiological quality of washing water for shredded lettuce

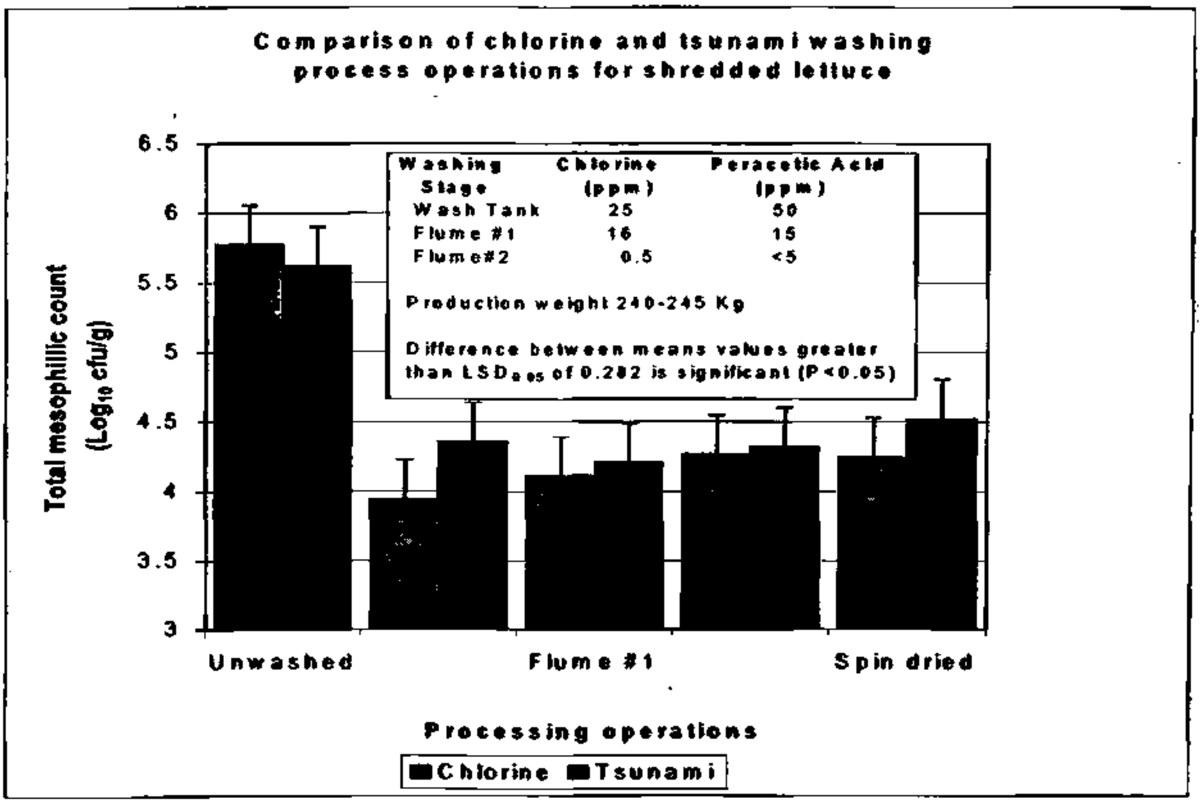
Processing operation (shredded lettuce production weight in kg)	Chlorine washing water (cfu/100mL)[1]	Tsunami washing water (cfu/100mL)[1]
Wash tank (pre-wash 0 kg)	<1	28
Wash tank (110-130 kg)	2	1730
Wash tank (240-245 kg)	1	2600
Wash tank (350-380 kg)	<1	3560
Flume #1 (240-245 kg)	NT[2]	2000
Flume #2 (240-245 kg)	NT	1120

[1]Average, n=2

[2]NT =Not Tested

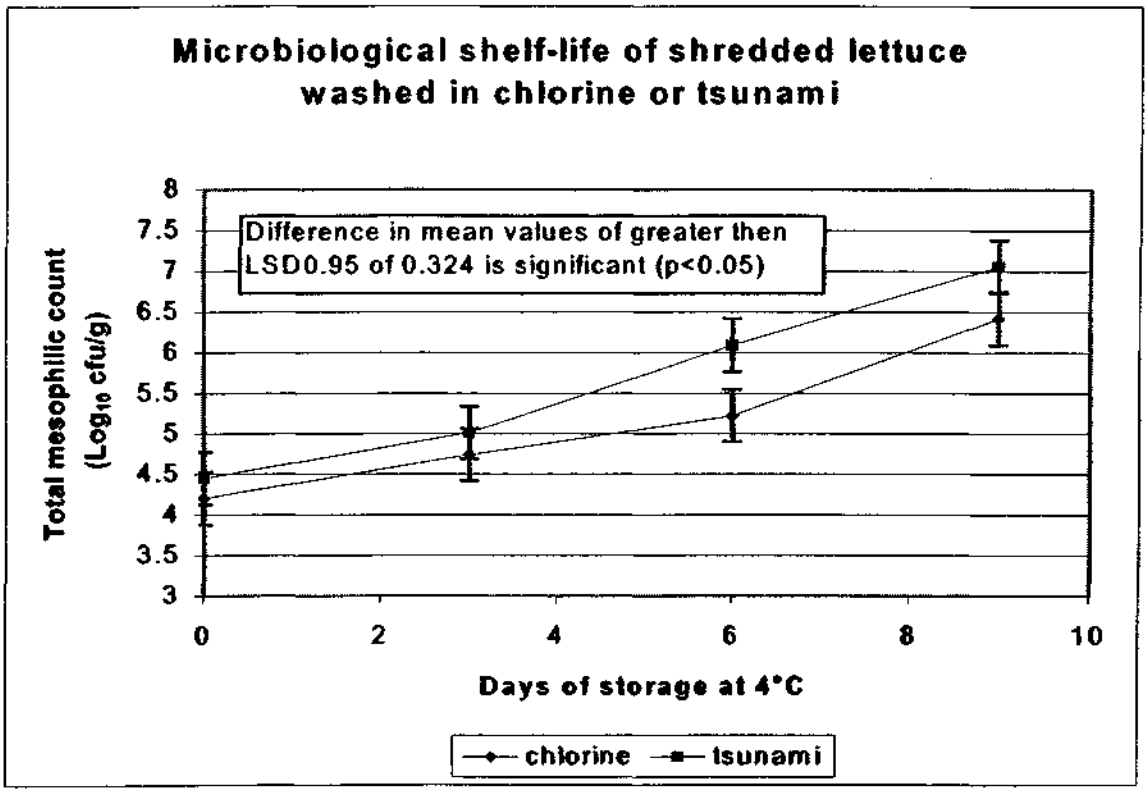
A comparison of processing operations for the chlorine and tsunami systems indicated a significant difference ($p=0.05$) in the total mesophilic count on product collected from the wash tank. There was no significant difference ($p=0.05$) between product samples collected from the 2 flumes. The microbiological count on the tsunami washed product did not significantly change following flume rinsing. There was a significant increase in the microbiological count on the chlorine washed product following flume rinsing, however following spin drying the average counts declined. The significant difference in the spin-dried product, which was not apparent with the more extensive sampling of product throughout the processing shift (see Figure 1). Therefore this result was discounted due to the limited sample size.

Figure 3:



The shredded lettuce was vacuum packaged in 2.5 kg bags (PD???, Cryovac) then stored at 4°C. The chlorine and Tsunami washed products retained a very good appearance after 9 days of storage, without any browning and a slight loss in crispness. The package atmosphere for both products equilibrated at a range of 0.2-1.5 % oxygen and 15-18% carbon dioxide. Immeadiately after washing and at 3 days of storage there was no significant difference in the total mesophillic counts. However, following 3 days of storage the total mesophillic population grew faster on the Tsuanmi treated product attaining significantly higher counts then the chlorine washed product on days 6 and 9.

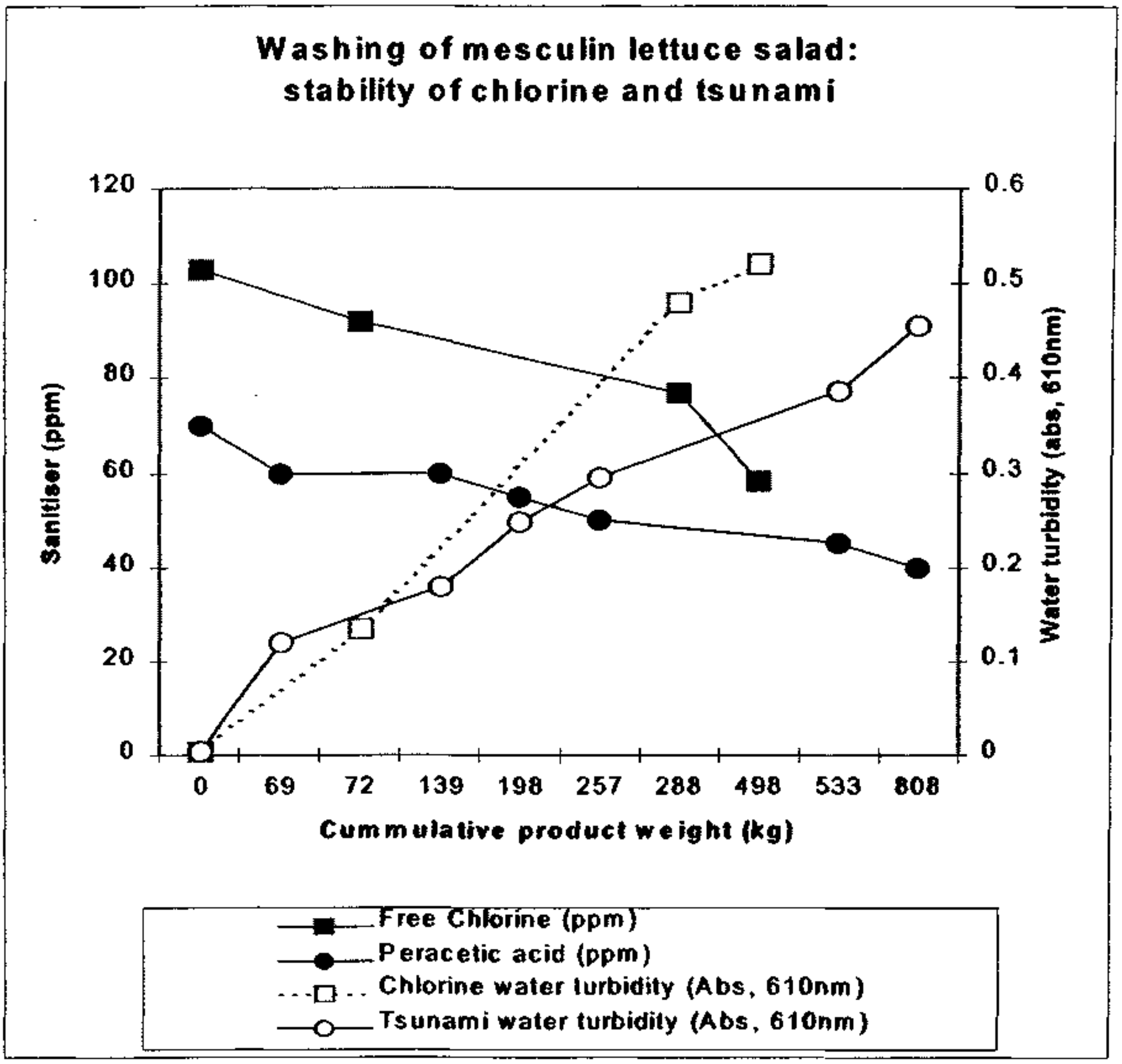
Figure 4:



Comparison of washing mesculin lettuce salad with chlorine or Tsunami treated water:

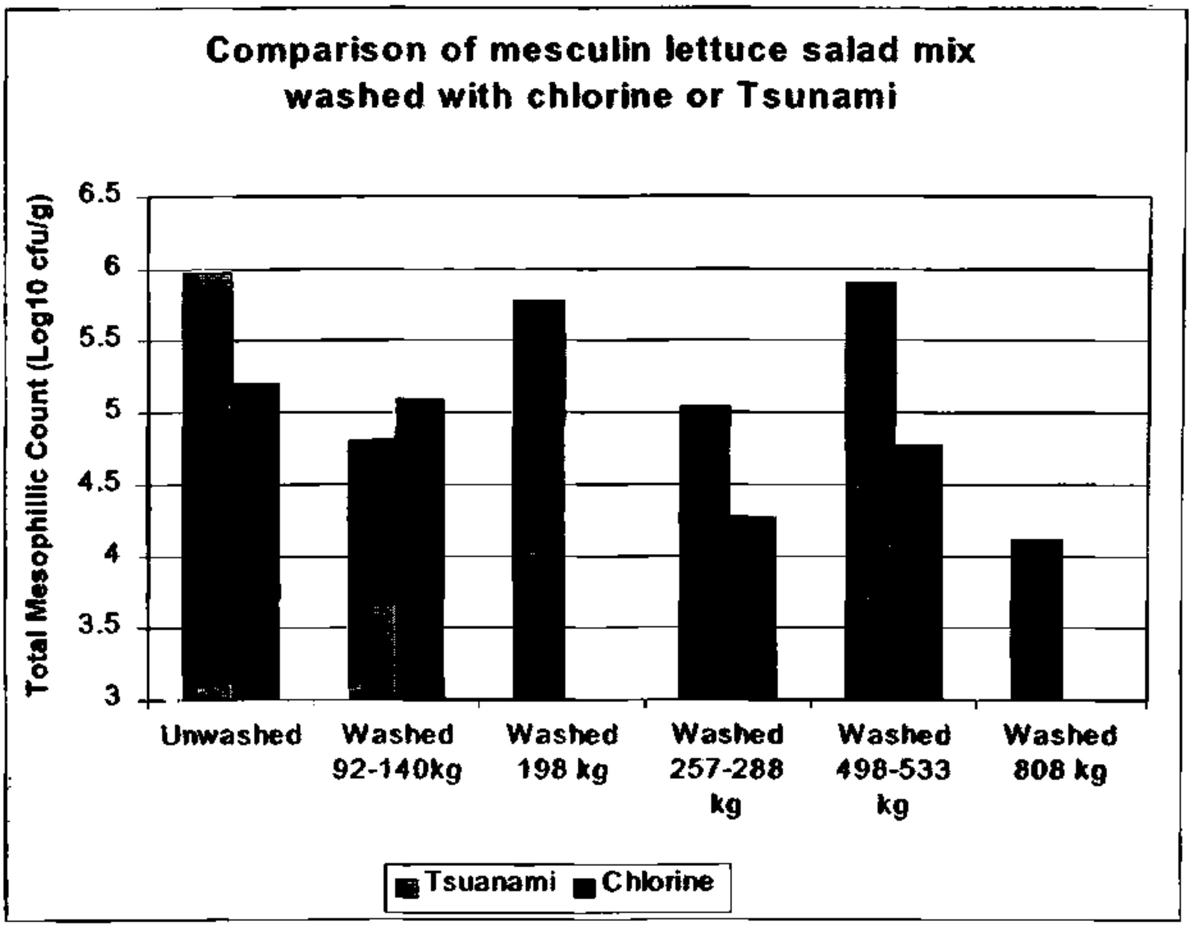
The initial level of free chlorine at 103 ppm rapidly declined throughout the processing shift to 60 ppm, following the production of about 500 kg of lettuce. Throughout this period the pH remained between 5.63-6.03. In comparison with Tsunami the peracetic acid commenced at 70 ppm and decline at a slower rate to 40 ppm, following the production of about 800 kg of lettuce (see Figure 5). This corresponded to a % decline of chlorine and Tsunami sanitiser of 43% and 35% respectively, based on 500kg of washed mesculin lettuce salad. The organic load in both washing systems was measured using ATP-bioluminescence. The levels were less then 1000 RLU, which was comparatively much lower then levels in excess of 1,000,000 for shredded lettuce. Less organic material is released from the mesculin lettuce because there is only a single cut on the main stem. The mesculin lettuce leaves have a high soil loading, therefore turbidity (Abs, 610nm) was used as an indicator of water quality. The turbidity of the washing increased rapidly for both washing systems during the processing shift (see Figure 5). The high organic load released from the shredded lettuce caused a more rapid depletion of both the sanitisers then the high soil loading present when washing the mesculin lettuce salad. No acetic acid (a by-product of peracetic acid) or hydrogen peroxide gas was detected above the washing tank

Figure 5



The microbial reduction achieved with both chlorine and Tsunami washing of mesculin lettuce salad was sparodic. In some instances during the production shift the microbial reduction was minimal where as at other times a 1-2 log reduction was achieved. There was a large variability in product counts at each sampling period. This is likely to be a consequence of the non-uniformity of the product, which consisted of between 9-13 different lettuce types. Therefore it was impossible to conclusively establish a comparison between the sanitisers (see figure 6).

Figure 6



There was a carry over of chlorine on the product into the 1st and 2nd water rinsing flumes, attaining free chlorine levels of 30 ppm and 6 ppm respectively. The carry over of chlorine in small quantities was favourable for maintaining the microbiological quality of the product (see Figure 7) . The total mesophillic count in the 1st and 2nd water rinsing flumes remained low (see Table 2), despite the soil loading increasing in water turbidity (measured by Abs, 610nm) to an average of 0.361 and 0.202. There was also a carry over of Tsunami into the 1st and 2nd water rinsing flumes, attaining peracetic acid levels of 15 ppm and <5 ppm respectively (see Figure 3). The total mesophillic counts in the Tsunami treated washing water were considerably higher (88-292 cfu/100mL), then for the chlorinated washing system (see Table 2).

Table 2: Microbiological quality of washing water for mesculin lettuce salad

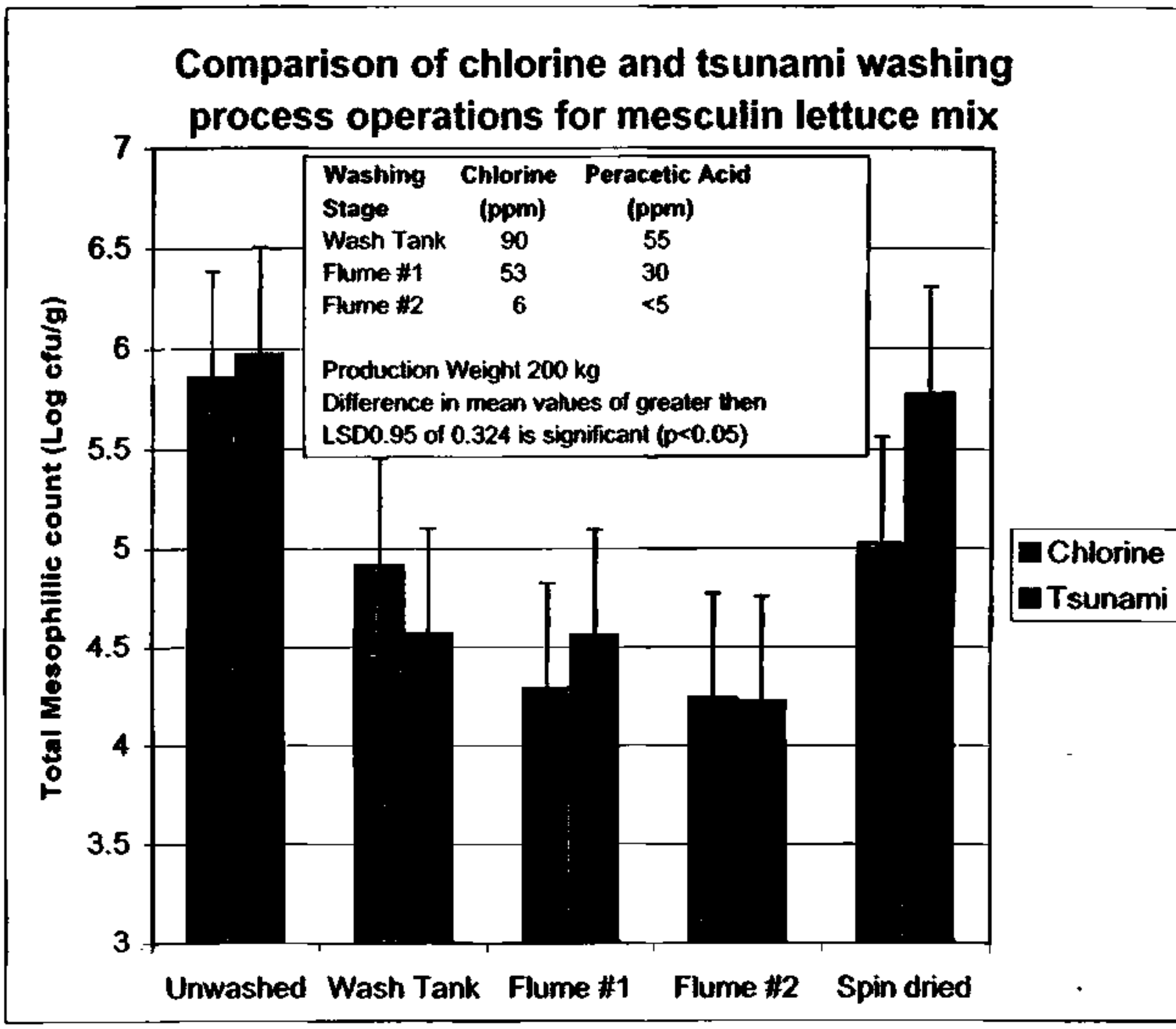
Processing operation	Chlorine washing	Tsunami washing

(shredded lettuce production weight in kg)	water (cfu/100mL)[1]	water (cfu/100mL)[1]
Wash tank (pre-wash 0 kg)	<1	245
Wash tank (69-72kg)	1	7,600
Wash tank (257-288 kg)	1	11,600
Wash tank (498-533 kg)	1	31,000
Flume #1 (240-245 kg)	<1	18,500
Flume #2 (240-245 kg)	2	16,800

[1]Average, n=2

There was a significant difference between the unwashed and product samples collected from the wask tank and the 2 rinsing flumes for both the chlorine and Tsunami washing systems (see Figure 7). Flume rinsing appeared beneficial for the microbiological quality of the product for both the chlorine and Tsunami washing systems, reducing the average total mesophilic population. A significant decrease in the microbiological count on the chlorine washed product occurred following flume rinsing. The total microbiological count for the spin dried product was on average higher then product sampled from the washing tank or flumes. This maybe due to the residual effect of sanitiser on the product with samples taken directly from the washing tank or flumes. There was a large variability in washed product (sampled after spin drying) throughout the processing shift (see figure 6). Increased variability due to the non-uniformity of the product (9-13 different lettuce types) has made it difficult to conclusively establish a comparison between the process operations.

Figure 7:



Shelf-life was not completed for mesculin shredded lettuce.

DISCUSSION

Tsunami was a more stable sanitiser then chlorine when there was a high organic and soil load. The microbiological reduction achieved by both sanitisers was not significantly different. There was no chlorine, acetic acid or hydrogen peroxide gases detected directly above the washing tank. Therefore using this style of washing system there was no occupational health and safety concern in relation to sanitiser and by-product gas-off. The product in the washing tank is mixed using large water jet manifold system with minimal aeration. Evaluation is also required for a system that uses air jets or creates aeration at the water surface that may result in sanitiser gas-off.

A larger proportion of both sanitisers was transferred into the rinsing flumes with the mesculin lettuce salad compared with the iceberg salad. Due to the larger size and shape of the mesuclin lettuce they trap more of the sanitised washing water which was transferred to the rinsing flume.

Mesculin flume rinse had a microbial reduction trend probably due to large level of sanitiser carry over compared with the shredded lettuce product.

The appearance of shredded iceberg lettuce following chlorine or Tsunami washing and storage at 4°C for 9 days was very good, without browning, but with a slight loss in crispness. The controlling of browning is also dependent on using permeable packaging to attain a modified atmosphere. The microbiological counts on the Tsunami washed product was significantly higher at the end of shelf-life.

CONCLUSIONS

- **Tsunami and chlorine provided similar product sanitation**
- **Tsunami was more stable then chlorine during high organic washing load.**

FURTHER WORK

- **Shelf-life of mesculin lettuce salad**
- **On-line monitoring and dosing control for Tsunami**

Milestone #9: Preliminary investigation of ultrasonic washing on minimally processed vegetables

EXECUTIVE SUMMARY

Preliminary trials were conducted to evaluate the suitability of ultra-sonicated washing on shredded lettuce based upon microbiological efficacy. Small scale trials were conducted in a 20 litre sonication tank with batches of shredded lettuce. Washing shredded lettuce in sonicated water achieved a significantly ($p<0.05$) greater reduction in the total mesophillic count on the shredded lettuce, compared with washing in water without sonication. The use of sonicated water achieved a total microbial reduction in the range of 0.61 to 1.26 Log on shredded lettuce for a period of 2 to 5 minutes. This was increased to a 1.7 Log reduction on average for an extended sonication period of 12 minutes. However, the longer period of application is not practical for commercial application and resulted in visible damage to the lettuce surface. Further trials combining sanitisers with sonication were conducted for 2 minute periods. Batch washing trials demonstrated that the reduction in the total mesophillic count on the shredded lettuce was significantly ($p<0.05$) greater using sonicated chlorine or Tsunami treated water compared with the non-sonicated application of both saintisers. A continuous washing system was operated by recycling the sonicated washing water through a heat exchanger to maintain the water temperature at 3-4°C. The washing water in the sonication tank was replaced approximately every 5 minutes. Using this system several batches of shredded lettuce were washed in sequence. The organic level in the washing water rapidly increased (as measured by total ATP levels) with the consequence of a complete reduction in the effect of sonication. The sonication system was incompatible for washing water with a high organic load . Further trials will be conducted with a more advanced sonication system which is designed to adapt for varying loads in the sonication washing tank. Scalime have made an expression of interest in the continuance of this research because it represents a potential physical alternative to chlorine washing, or could be used in combination to enhace existing systems or reduce the required levels of chlorine. Further preliminary trials are required to provide sufficient performance data to justify the design and construction of a pilot-scale system suited for fresh-cut operations.

INTRODUCTION

Ultrasonication can create ultrasound waves (UW) generated by mechanical vibration frequencies higher than 15kHz. When these waves are propagated in a liquid they produce alternating compression and expansion cycles. During the expansion cycle, UW make small air bubbles grow in a liquid. When they reach a volume at which they can no longer absorb more energy, they implode violently. This phenomenon is known as cavitation. During implosion, very high temperatures and pressures are reached inside the bubbles. This effect is thought to be the main reason for the bactericidal effect.

The effect of a santising agent (ie. chlorine) is less effective on bacteria that are attached and trapped on the product surface (ie.formation of biofilms). It has long been established that UW can break up bacterial clumps of bacteria that naturally occur in milk (stone and Fryer, 1984), including the removal of bacteria from milk bio-films on metal surfaces (Daufin and Saincliviert, 1967). UW was demonstrated to improve surface sterilisation of poultry skin in combination with chlorine (Lillard, 1994, Sams and Feria, 1991).

The application to fresh-cuts would utilise ultrasonic cleaning systems normally used for cleaning equipment. This requires a power supply for generating ultrasonic energy and immersible transducers which are mounted in the washing tank to optimise the distribution of UW.

PROJECT AIM

Evaluate the suitability of using ultra-sonication for the washing of fresh-cut vegetables, based upon microbiological and organoleptic quality.

METHODS

Description of ultrasonication washing system:

An ultrasonication system was leased from Ultrasonics Consolidated (P/L) with the following specifications.
Volume: 15 litres
Frequency output:40 MHz

Temperature control set up: 3-4°C
Total volume: 40 litres (including heat exchanger)
Flow rate: 300 litres per hour

Product description:

Shredded iceberg lettuce of about 1cm in width.

Microbiology

Products were sampled in triplicate for microbiological analysis, 20 gram portions were homogenised (Colworth Stomacher) in 180 mL of 0.1 % bacteriological peptone (Oxoid), then spiral plated in duplicate on plate count agar (Oxoid) and incubated for 2 days at 30°C.

Water samples were filtered through TTC filter pad (Sartorious), then placed on a moistened nutrient pad and incubated for 2 days at 30°C.

Package headspace gas analysis

Gas analysis of the packaged headspace for carbon dioxide and oxygen content. Packaged headspace was analysed using a MAPtest 4000 equipped with an infra-red carbon dioxide analyser and zarconia sensor for oxygen

Statistical Analysis

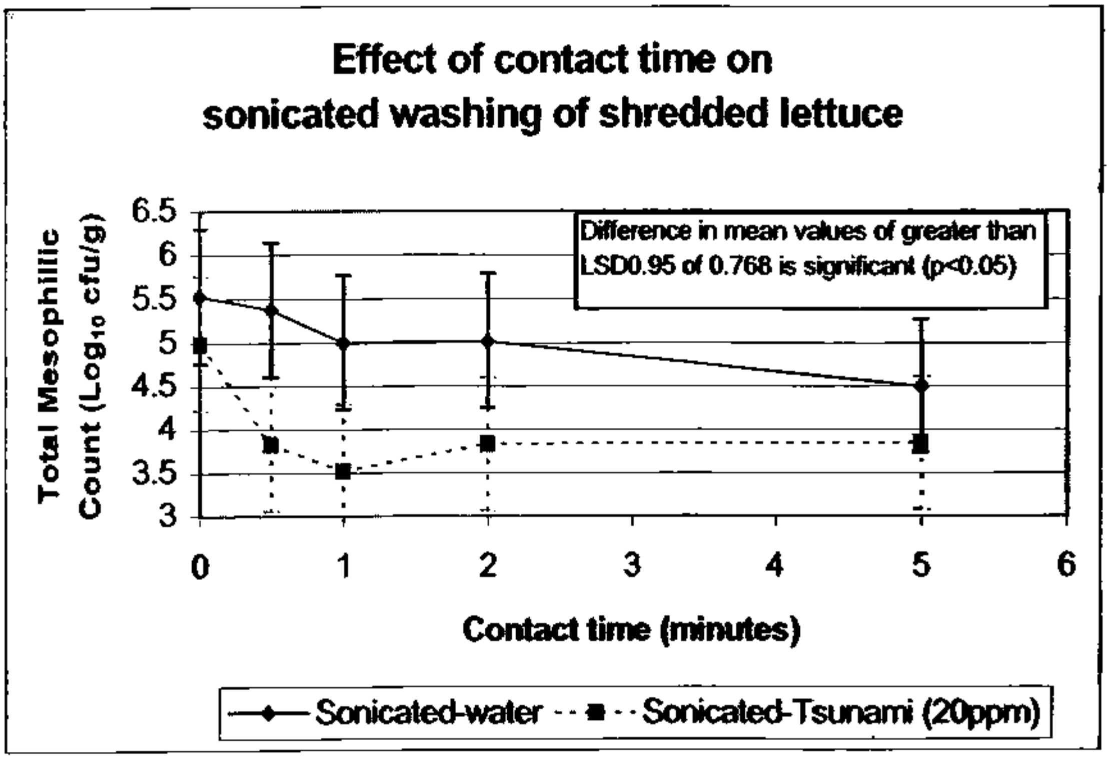
The mean of 3 replicate determinations are presented with respective standard deviation. Analysis of variance (single factor) was calculated using Genstat version 3.2 for calculation of least significant difference ($LSD_{0.95}$). Difference between mean values greater then $LSD_{0.95}$ is significant, at $p<0.05$.

RESULTS

Effect of contact time on sonicated washing

Washing the shredded lettuce in sonicated water for 30 seconds provided a marginal reduction in the total mesophillic count on the shredded lettuce. Approaximatley 0.5 log reduction was achieved with 1 to 2 minutes sonicated washing time. Greater than a 1 log reduction was achieved with a treatment of 5 minutes (see Figure 1). Prolonged treatment for 12 minutes enabled a microbiological reduction of 1.5 Log, but the lettuce tissue was visibly damaged, becoming translucent (see Figure 2).

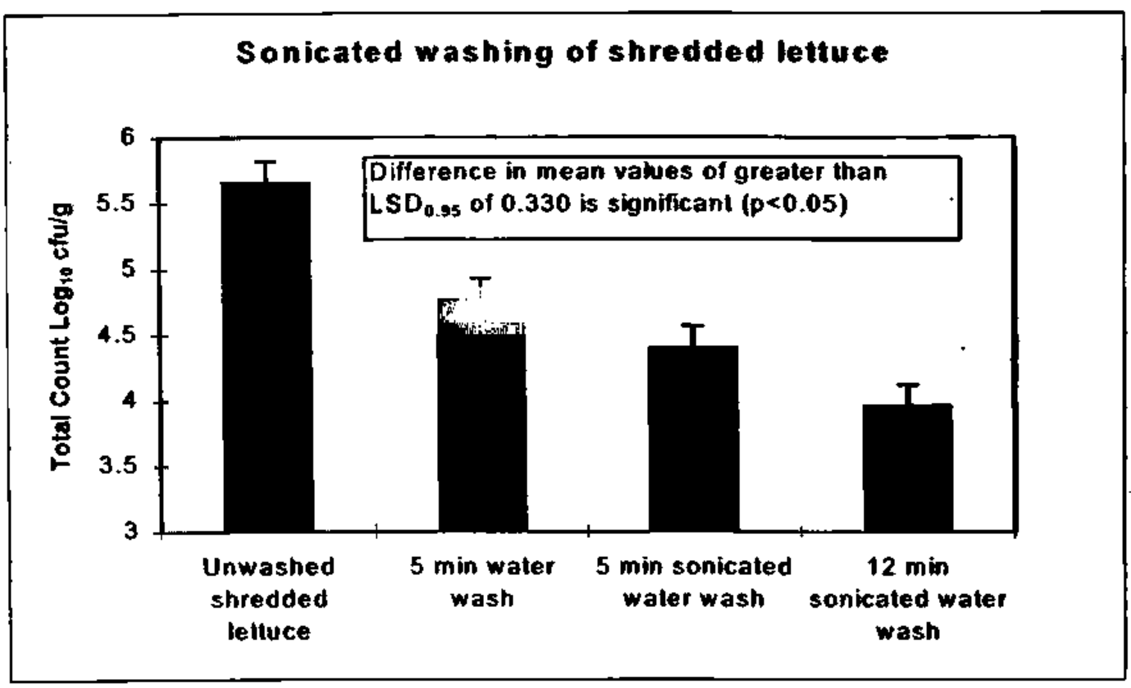
Figure 1.



The total mesophillic count on the shredded lettuce washed in sonicated water was significantly lower than that washed in water without sonication (see Figure 2).

Investigation of the effect of contact time when washing shredded lettuce in sanitised-sonicated water demonstrated that a significant reduction in the total mesophillic count was achieved with a contact time of only 30 seconds. There was no additional benefit with extending the contact time beyond 1 minute (see Figure 1).

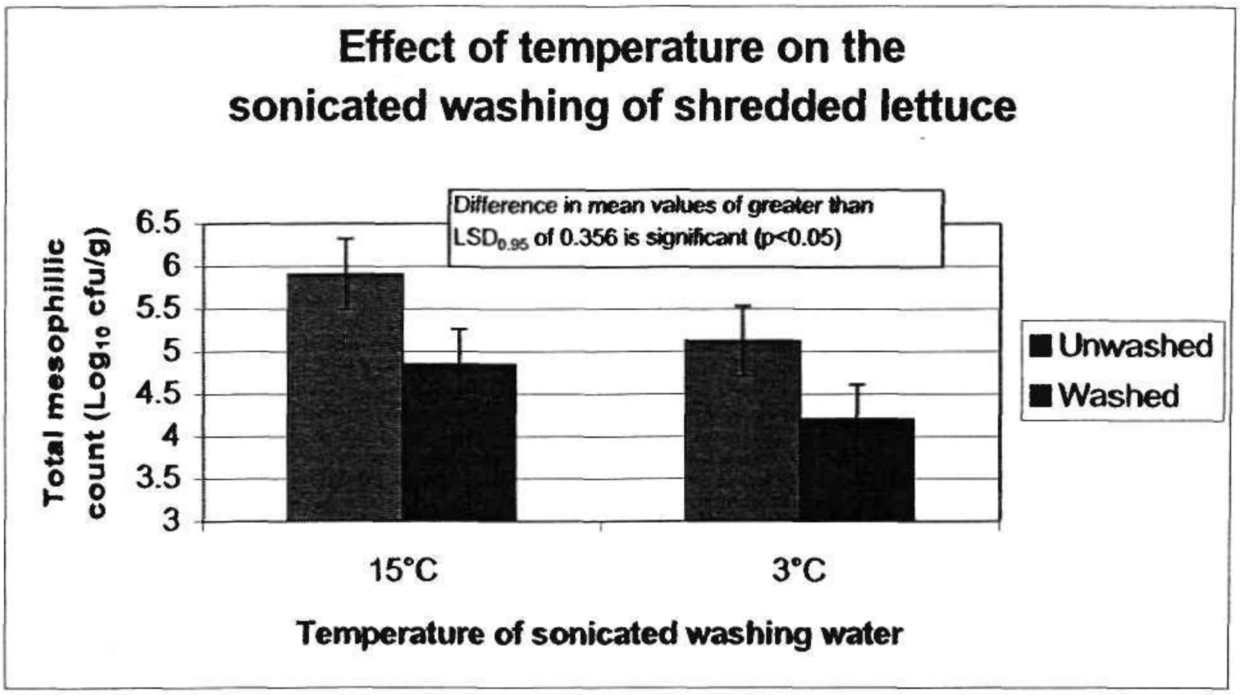
Figure 2:



Effect of temperature on sonicated washing

The sonicated washing water was cooled by recycling it through a heat exchanger and returning it to the washing bath. The reduction in total mesophillic count on the shredded lettuce was comparatively similar at both 15°C and 3°C, achieving 91% and 87% respectively (see Figure 3).

Figure 3:



Sanitised sonication washing system

Washing shredded lettuce in sonicating water achieved a significant reduction in total mesophillic count compared with water washing (see Figure 4). A sonicating water wash was comparable with washing the shredded lettuce in Tsunami treated water at 10 ppm peracetic acid. Sonicating the Tsunami treated washing water achieved a significant improvement in the reduction of the total mesophillic count compared with water washing (see Figure 4). The sanitation performance of chlorine was also significantly improved by sonicating during the washing of shredded lettuce (see Figure 5).

Figure 4:

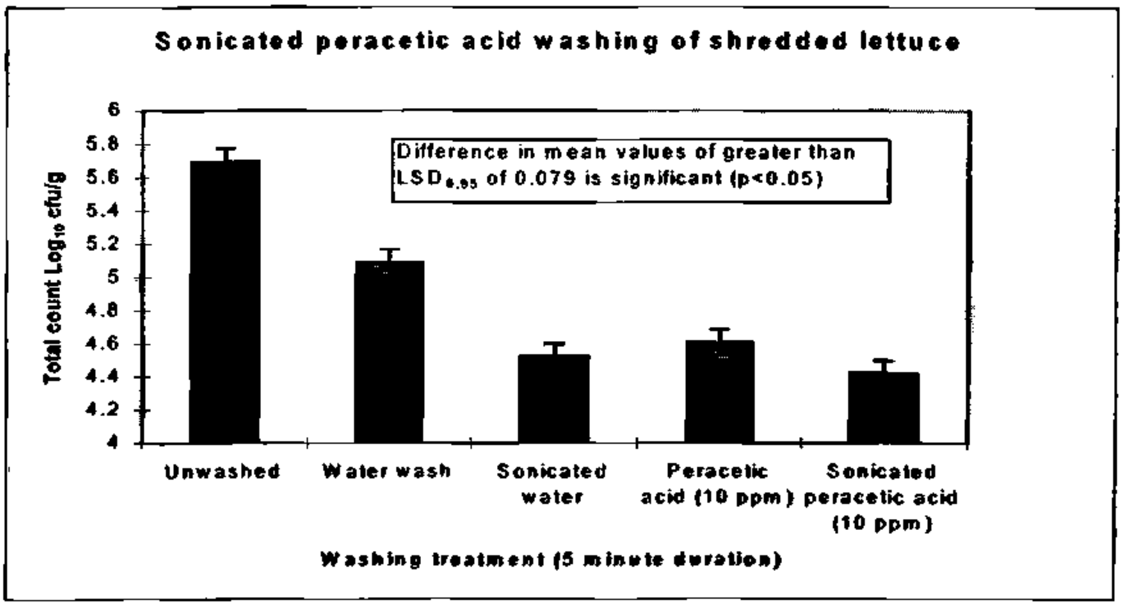
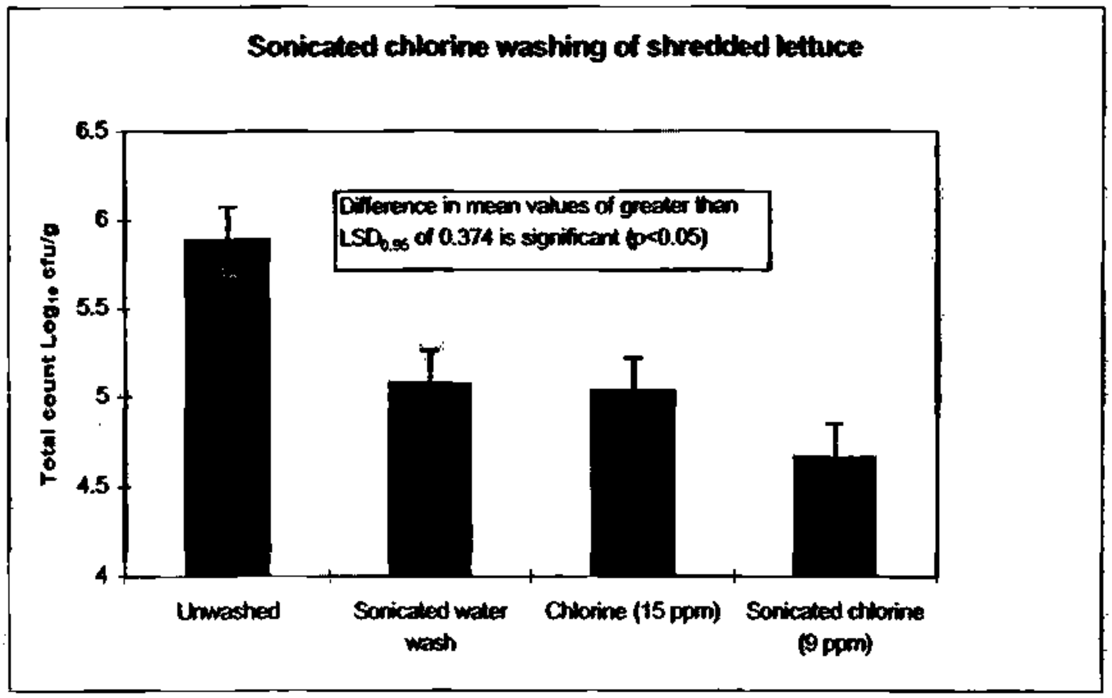


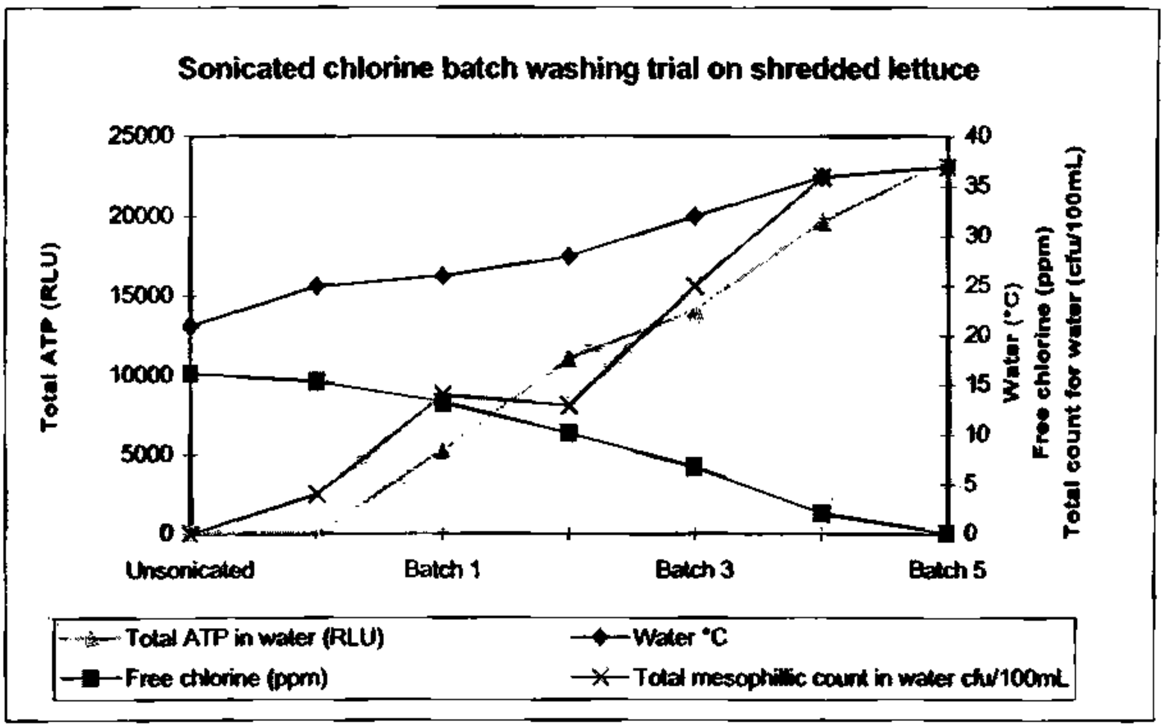
Figure 5



Batch washing trial

A batch washing trial was conducted with sonicating chlorinated washing water. During sonication the temperature increased from 25°C to 37°C (see Figure 6). Without water recycling or screening the organic load increased in the sonication bath with the addition of each batch of lettuce. The total mesophillic count in the washing water increased to 37 cfu100mL. The chlorine levels were rapidly reduced to less then 1 ppm, due to gass-off with the raised water temperature (see Figure 6). Consequently, water recycling and temperature control is required to reduce sanitiser gass-off, microbial growth and product respiration rate.

Figure 6:

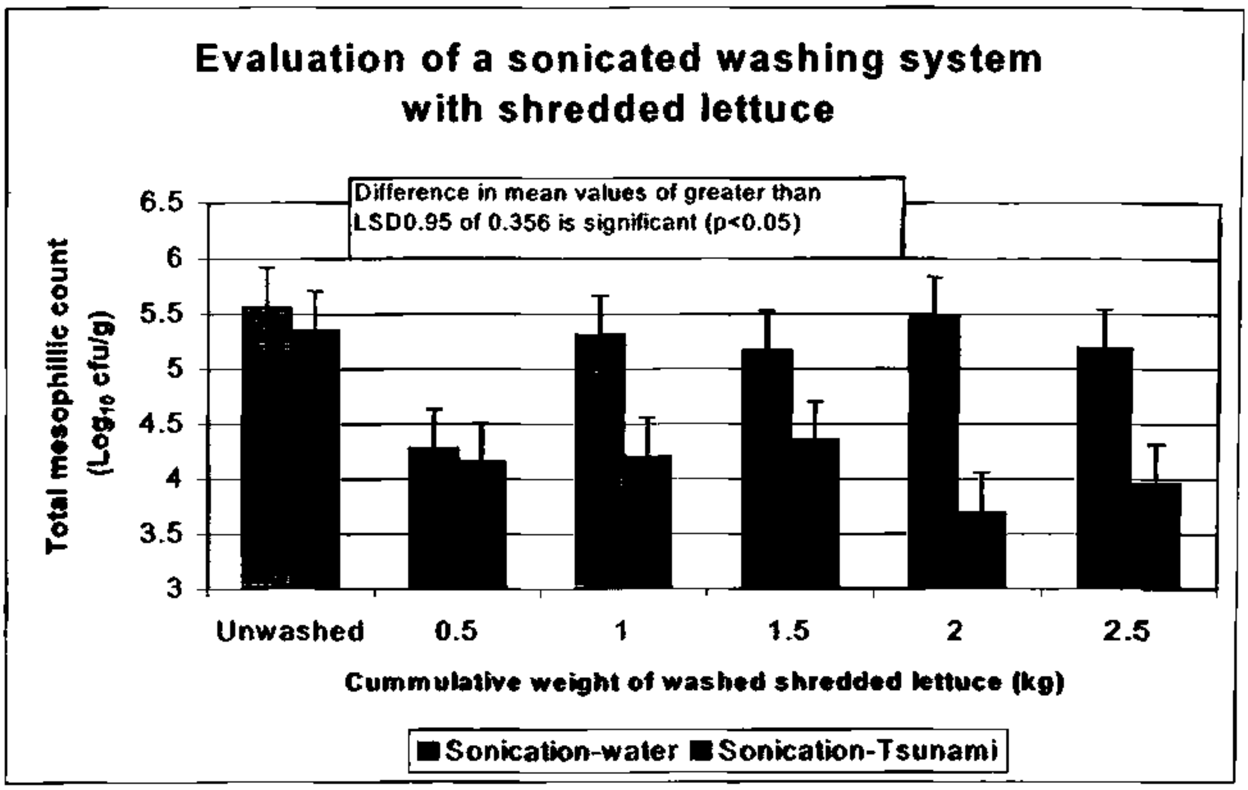


Water recycling and temperature control

The temperature of the water in the sonicating washing bath (15 litres) was held between 2.9-3.7°C by pumping the water through a heat exchanger. The total volume of the washing bath was recycled approximately every 5 minutes.

When washing the first batch of shredded lettuce in the sonicating water bath a significant reduction in the total mesophillic count was achieved. For the subsequent batches of shredded lettuce there was no significant reduction in the total mesophillic count. The ineffectiveness of the sonication with subsequent batches maybe due to the effect of water recycling reducing the gassing-off and the accumulation of the organic load. When using Tsunami (20 ppm) treated water in the sonication bath a significant reduction in the total mesophillic count was achieved throughout the trial (see Figure 7). The concentration of peracetic acid was maintained during the washing trial at 20 ppm.

Figure 7:



Two-stage washing combining sonication, emulsifying agent and chlorine or Tsunami

The average reduction of the total mesophillic count on shredded lettuce was improved (but not significantly, p=0.05) for chlorine and tsunami by conducting ultrasonication for 2 minutes with an emulsifying agent (Dipersa-pak, Hydroc™), followed by a sanitised wash with chlorine or Tsunami for 2 minutes (see Figure 8). The total mesophillic counts on shredded lettuce washed in sonicated water containing an emulsifying agent and washed in Tsunami treated water was significantly lower than the unwashed shredded lettuce during storage for 7 days at 4°C.

Figure 8:

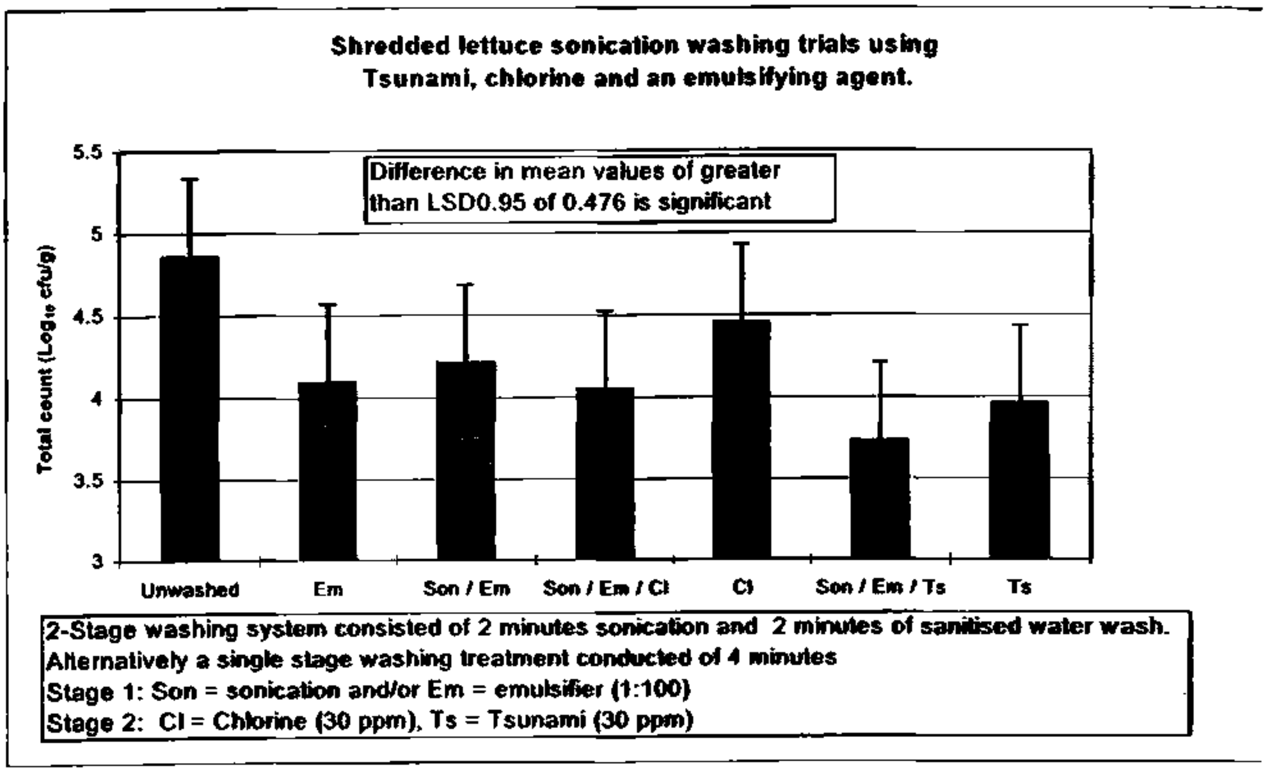
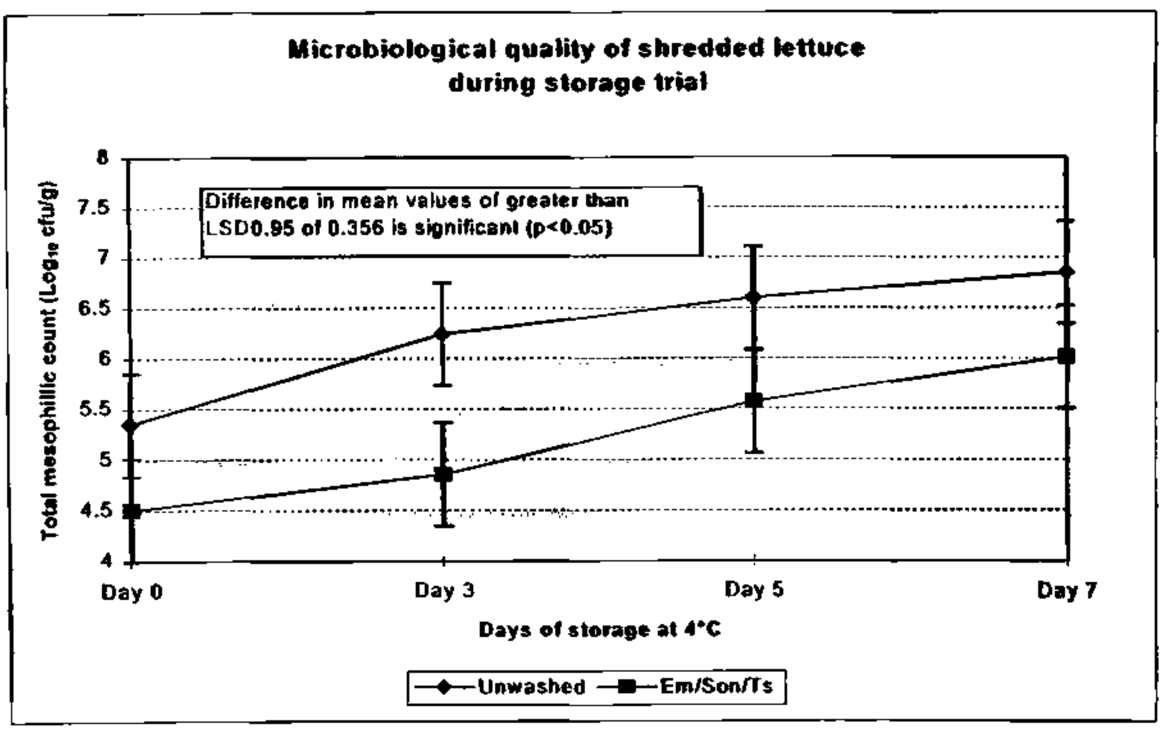


Figure 9:



DISCUSSION

The sanitation of a single batch of shredded lettuce with sonicating water achieved a significant reduction in the total mesophillic count on shredded lettuce. The reduction in the total mesophillic count using chlorine and Tsunami was significantly improved with the sonication of the washing water.

To meet the requirements of fresh-cut processors the sonication system was modified to include water recycling through a heat exchanger to control the temperature of the washing water, while several batches of shredded lettuce were washed.

For the initial batch of shredded lettuce the ultra-sonication was effective in significantly reducing the total microbiological count on the shredded lettuce. However, for subsequent batches of shredded lettuce ultra-sonication was not effective. This may be an effect of the increased organic load in the washing system as monitored by ATP levels. The recycled water could be screened and filtered to reduce the organic load accumulating in the washing system.

RECOMMENDATION

- Conduct further trials using a more advanced ultra-sonication system that can adapt to changes in the wash tank loading.
- Investigation of higher frequency levels to provide more precision in cleaning of intricate surfaces such as lettuce.

Milestone #10: Evaluation of ultrasonic washing for minimally processed vegetables

EXECUTIVE SUMMARY

Ultrasonic washing was evaluated on shredded lettuce, broccoli florets and carrot slices for chilled (4-5°C) and heated (40-50°C) washing applications. Chilled washing was used because it is standard practice for the fresh-cut industry. Heated washing was conducted because it is effective in deactivating browning enzymes.

The total viable reduction when washing in chilled water ranged from 0 to 0.5 Log cfu/g, this was increased to 0.6 to 1.0 Log cfu/g with the application of ultrasonics. Sanitation of the water with peracetic acid at 20 ppm resulted in a total viable reduction ranging from 1.3 to 1.5 Log cfu/g, which was comparable with 1.2 to 1.7 Log cfu/g reduction achieved with the application of ultrasonics. The ultrasonic washing with chilled water (4-5°C) was only marginally effective. Cavitation was not adequately achieved in the chilled washing water, the slight improvement observed in the reduction of the average total viable count for both sanitised and non-sanitised washing was not operationally significant (as established from industry consultation). The total viable count reduction by washing in heated water ranged from 0.3 to 1.4 Log cfu/g, this was increased to 0.7 to 1.8 Log cfu/g with the application of ultrasonics. Sanitation of the water with peracetic acid at 20 ppm resulted in a total viable reduction ranging from 0.5 to 1.8 Log cfu/g, which was increased up to 2.3 Log cfu/g reduction with the application of ultrasonics. The range in the results varied depending on treatment time, ultrasonic operational function and the inherent variation encountered when determining microbiological populations on vegetable produce.

When compared with the industry practice of chilled washing, the application of sanitised ultrasonic washing at 50°C was more effective in reducing the total viable population on shredded, broccoli florets and carrot slices, increasing the Log reduction from 1.1 to 2.2 Log cfu/g, 0.7 to 2.9 Log cfu/g and 1.5 to 2.2 Log cfu/g for each respective product. The reduction in the total viable population was due to the combined effect of heated washing water, sanitation and ultrasonics. Each individual treatment was also demonstrated to provide a greater reduction in the total viable populations.

Deterioration in the quality of the shredded lettuce was observed during storage when ultrasonic washing was performed at 50°C for greater than 1 minute, due to loss of crispness, flaccidity and moisture accumulation. The deterioration in product quality was avoided at 35°C, however this temperature was not effective in reducing the enzymatic browning. At 35°C the application of ultrasonics provided an increased reduction in the total viable number by 0.6 Log cfu/g. An ultrasonic washing application at 40°C for 1 to 1.5 minutes was considered most appropriate to provide the greatest total viable count reduction while still retaining good product quality. The visual quality of the broccoli florets was very good for 3 days of storage, but was unacceptable after 7 days at 4°C, due to excess moisture accumulation, softening and development of an off-odour. An ultrasonic washing application of 40°C for 2 minutes was considered most appropriate to provide the greatest total viable count

reduction while still retaining good product quality. The visual and microbiological quality of the carrot slices were both improved with the sanitised-heated ultrasonic washing, due to the reduction of whitening and delay in microbial growth during storage.

With an increasing organic load, a similar rate of decline in peracetic acid concentration was observed with and without ultrasonic application. When the peracetic acid had completely declined due to very high organic loading, the average reduction in total viable population was higher for ultrasonic washing.

Ultrasonic washing of minimally processed vegetables is not suited to chilled washing applications currently conducted by growers and fresh-cut processors. Ultrasonic washing could provide an alternative treatment process, which in combination with mild heat treatment and low-level sanitation to attain a greater reduction in the total viable count on produce, providing appropriate operating conditions are used to prevent deterioration in product quality.

INTRODUCTION

Ultrasonic washing involves generating ultrasonic sound waves by mechanical vibration frequencies greater than 15kHz. The sound waves produce alternating compression and expansion cycles, during which small air bubbles grow until they reach a volume at which they can no longer absorb more energy and implode violently. This phenomenon is known as cavitation.

During 1998-1999 a preliminary investigation into ultrasonic washing of minimally processed vegetables was conducted (submitted to HRDC March 1999, Milestone #5). This investigation demonstrated that the reduction in total viable count on shredded lettuce during sanitised washing (with chlorine and Tsunami) was significantly greater ($p<0.05$) with ultrasonic (25kHz) treatment. The ultrasonic washing was found to be ineffective with increasing organic load, which reduced cavitation. To overcome this limitation an advanced ultrasonic generator was sourced from Branson (US), for trials during 1999-2000 period. This system offered the additional benefits of (i) auto-tuning to compensate for changes in the loading in the washing tank, (ii) line/load regulation to compensate for changes in liquid level and temperature, (iii) variable power control of cavitational intensity allowing for matching to the application, (iv) selectable sweep frequency to reduce standing waves and improve ultrasonic distribution, and (v) power mode used for driving difficult to cavitate systems.

PROJECT AIM

Evaluation and development of ultrasonic washing applications and protocols for minimally processed vegetables.

METHODS

Description of ultrasonication washing system:

A Branson ultrasonic power supply (model 8500) with a frequency output 25 kHz, was used to drive a 15 litre ultrasonic washing tank with 12 in-built transducers. The ultrasonic washing system was leased from Ultrasonics Consolidated (P/L).

Temperature control of washing water

Heating of the washing water was conducted using a coil immersed in a heated washing tank and recirculated at 90 litres per minute. Washing water was chilled by recirculating through a plate heat exchanger connected to chilled water glycol system or pre-chilled in batch tanks within a chiller room.

Product description and preparation:

Washing trials were performed on the following products:

- The outer leaves of iceberg lettuce and the core was removed, then shredded (5mm in width).
- Broccoli florets were cut to size of approximately 5 grams, the main stem was discarded.

- Carrot slices prepared by abrasively peeling carrots and water rinsed to remove peel then sliced (4 mm in width). Carrot batons were processed in the same manner without slicing.

Product was pre-chilled in a cool room at 4°C for about 24 hours prior to processing.

Washing, de-watering and packaging

The product was immersed in the ultrasonicwashing tank for periods from 15 seconds to 5 minutes. Specific immersion times are detailed for each trial in the results section. Product was spin dried for 2 minutes, then 200 grams was packaged into 55 micron polyethylene blend film, with an oxygen transmission rate of 4000 cm^3/cm^2 of film/day.

Monitoring sanitiser levels and washing water chemistry

The peracetic acid component of Tsunami was measured using peracetic acid test strips (Merck P/L))

The pH and oxidative redox potential (ORP) of the washing water was monitored continuously using HP2AS controller (TPS P/L) and 90-Link software system (TPS P/L) for down loading the data. The pH, ORP and temperature compensation probe were inserted into a flume (2 litre volume) which the recirculated water passed trough prior to re-entering the washing tank.

Microbiological analysis

Products were sampled in triplicate for microbiological analysis, 20 gram portions were homogenised (Colworth Stomacher) in 180 mL of 0.1 % bacteriological peptone (Oxoid), then pour-plated or spiral plated with plate count agar (Oxoid) and incubated for 3 days at 30°C.

Water samples were filtered through TTC filter pad (Sartorious), then placed on a moistened nutrient pad and incubated for 1 days at 30°C. Tsunami was neutralised by 1 mL of 1.8% sodium thiosulphate added to the 100mL collection container prior to sampling the washing water.

Statistical Analysis

The mean of 3 replicates, $\pm$ standard deviation, are presented in graphical formats.

RESULTS

Effect of contact time and temperature

Chilled (4-8 °C) ultrasonic washing treatments:

Washing of the shredded lettuce in water alone provided a reduction of the total microbial population in the range of 0 to 0.5 Log cfu/g. A slight increase in the average log reduction of the total microbial population was achieved by ultrasonic treatment of the washing water, which provided a reduction in the range of 0.6 to 1.0 Log cfu/g (see Figure 1). The larger microbial reduction was achieved with using high sweep, high density and amplitude ultrasonic treatments (see Figure 5 & 6). The stated ranges, also included data from ultrasonic contact time trials ranging from 15 seconds to 8 minutes (not presented in graphs).

Chilled (4-8 °C) sanitised ultrasonic washing treatments:

Washing of the shredded lettuce in Tsunami treated water (20 ppm peracetic acid) provided a log reduction of the total microbial population in the range of 1.3 to 1.5 Log cfu/g. Washing of the shredded lettuce in sonicated Tsunami treated water (20 ppm peracetic acid) provided a reduction of the total microbial population in the range of 1.2 to 1.7 Log cfu/g (see Figure 1, 5 & 6).

Heated (45-50 °C) ultrasonic washing treatments:

Washing of the shredded lettuce in heated water provided a reduction of the total microbial population in the range of 0.3 to 1.4 Log cfu/g. The higher microbial reduction was achieved using a treatment time of 2 minutes. Heated (45-50°C) ultra-sonic treatment time in excess of 90 seconds resulted in unacceptable damage to the shredded lettuce, resulting in transparent edges 9ie. water logged appearance) and early softening during storage. An increase in the log reduction of the total microbial population was achieved by ultrasonic treatment of the heated washing water, which provided a reduction in the range of 0.7 to 1.8 Log cfu/g. The larger microbial reduction was achieved using a 2 minute treatment time, high sweep, and amplitude ultrasonic treatments (see Figure 5). The microbial reduction with heated sanitised washing water was increased at most by 0.7 Log cfu/g.

Heated (45-50 °C) sanitised ultrasonic washing treatments:

Washing of the shredded lettuce in heated sanitised (Tsunami, peracetic acid 20ppm) water provided a reduction of the total microbial population in the range of 1.3 to 1.8 Log cfu/g. The higher microbial reduction was achieved using a treatment time of 2 minutes. An increase in the average log reduction of the total microbial population was achieved by ultrasonic treatment of the heated washing water, which provided a reduction in the range of 0.5 to 2.3 Log cfu/g. The larger microbial reduction was achieved using a 2 minute ultrasonic treatment. The microbial reduction with heated sanitised washing water was increased on average by 0.6 Log cfu/g and 0.8 Log cfu/g for treatment times of 1.5 and 2 minutes respectively.

Comparison of heated Tsunami washing of shredded lettuce with ultrasonic washing for the same conditions resulted in an increased average reduction in the total viable population for 1.5 minute (1.5 and 2.2 Log respectively), and 2 minute washing periods (1.8 and 2.3 Log respectively) (see Figure 2).

Comparison of heated Tsunami washing of broccoli florets with ultrasonic washing for the same conditions resulted in a comparable average reduction in the total viable population for a 1 minute washing period (1.11 and 1.04 Log cfu/g respectively), a marginal increase at 2 minutes (0.95 and 1.46 Log cfu/g respectively) washing time, and comparable reduction at 5 minutes (1.34 and 1.40 Log cfu/g respectively) see Figure 3).

The heated (50°C) sanitised washing of carrot batons in 20 ppm Tsunami for an extended period (from 1 minute to 5 minutes) resulted in an increase in the average reduction in total viable population (see figure 2). Comparison of heated Tsunami washing of carrot batons with ultrasonic washing for the same conditions resulted in a comparable average reduction in the total viable population for a 1 minute (1.14 and 1.17 Log cfu/g respectively), 2 minute (1.14 and 1.17 Log cfu/g respectively) washing period, and a marginal increase at 5 minutes (1.38 and 1.61 Log cfu/g respectively) (see figure 4).

Operational ultrasonic functions

The average reduction in total viable population was marginally higher for both chilled (7°C) and heated (50°C) washing when using the high sweep frequency-operational function (see Figure 5). In comparison with the other operational functions and taking into consideration the standard deviation of the Log reduction in the total viable population, the operational settings for standard ultrasonic (0.8 & 1.78 Log cfu/g reduction, at 7°C and 50°C respectively), high-sweep (0.94 & 1.88 Log cfu/g) and power mode (0.62 & 1.62 Log cfu/g) were considered comparable. A similar observation was also observed for chilled sanitised ultrasonic washing with a marginally higher average reduction in total viable population (see Figure 6).

Effect of organic load

Increased the organic load in the washing water by washing successive batches of shredded lettuce resulted in the depletion of the peracetic acid concentration (from 30 to 0 ppm) under ultrasonic and non-ultrasonic washing conditions at a similar rate (see Figure 7). During this period the pH of the washing water at commencement was 3.26 and 3.28 for ultrasonic washing and non-ultrasonic washing respectively, which rose to 3.63 and 3.54 respectively after the washing of 6 kg of shredded lettuce. The average microbial reduction declined for Tsunami washing (without ultra-sonication) from 2.25 to 1.5 Log with the depletion of Tsunami. When the Tsunami was depleted during ultrasonic washing, the average microbial reduction remained higher than 2 Log cfu/g (see Figure 7). During the washing period the total count in the water increased approximately 10-fold, with and without ultrasonics (see Figure 8)

Comparison of industry practice and ultrasonic washing system

Ultrasonic washing was conducted in heated sanitised (Tsunami, 30ppm peracetic acid) water at 50°C for 1.5 minutes with shredded lettuce and 5 minutes for broccoli florets and carrot slices. This was followed by a 1 minute rinse in chilled sanitised (Tsunami, 5ppm peracetic acid) water at 4°C. A comparison was made with a 2 step chilled sanitised washing procedure to replicate current industry practice. This involved, chilled sanitised (Tsunami, 30ppm peracetic acid) water at 5°C for 1.5 minutes with shredded lettuce and 5 minutes for broccoli florets and carrot slices. This was followed by a 1 minute rinse in chilled sanitised (Tsunami, 5ppm peracetic acid) water at 4°C. The average microbial reduction was higher with heated sanitised ultrasonic washing compared with the typical industry practice of chilled sanitised washing for shredded lettuce, broccoli florets and carrot slices (see Figure 9).

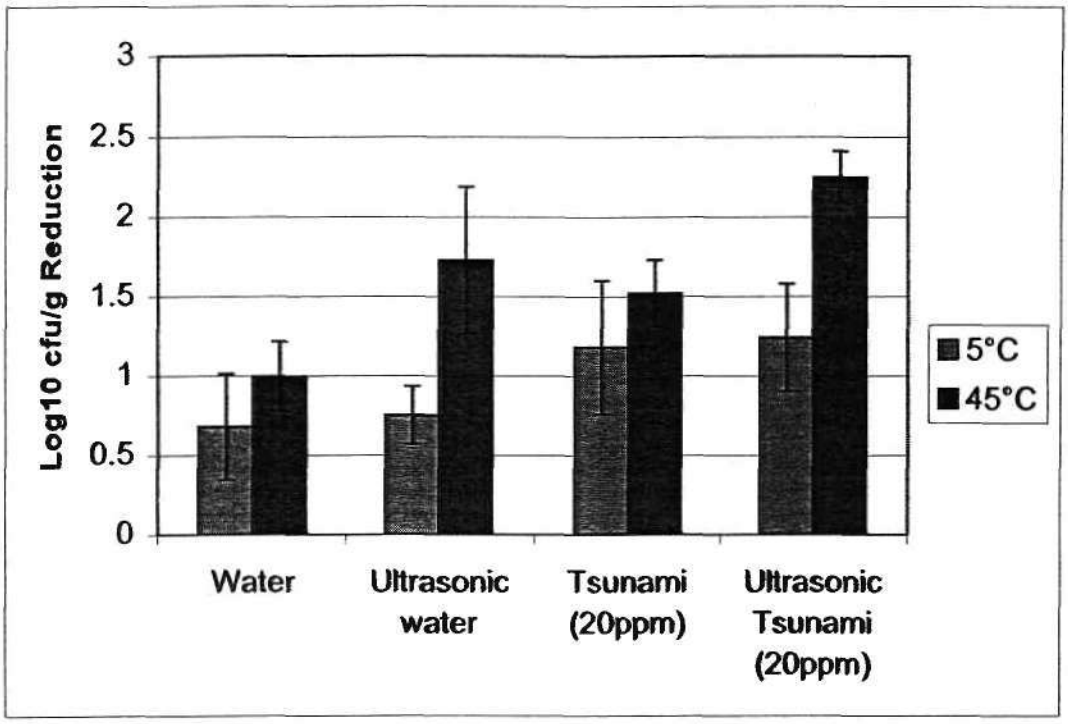
Microbiological shelf-life:

The total viable population on both chilled-sanitised washed and heated-sanitised ultrasonic washed shredded lettuce both grew rapidly from 5.1 and 3.5 to 7.4 and 7.8 Log cfu/g respectively in 10 days after storage at 4°C (see Figure 10). The total viable population on the chilled-sanitised washed broccoli grew rapidly from 4.8 to 6.5 Log cfu/g within 3 days, the maximum population of approximately 7.0 Log cfu/g, which was reached within 7 days. The total viable population on the heated-sanitised ultrasonic washed broccoli florets grew slightly in 3 days from 2.7 to 3.0 Log cfu/g, then grew more rapidly attaining 5.8 Log cfu/g after 10 days (see Figure 11). The total viable population on the chilled-sanitised washed carrot slices remained stable at 2.8 Log cfu/g for 3 days then grew rapidly to 6.5 Log cfu/g after 10 days. The total viable population on the chilled-sanitised washed carrot slices remained stable at 2.0-2.1 Log cfu/g for 3 days then grew slowly from to 2.8 cfu/g after 7 days, then grew rapidly to 6.5 Log cfu/g after 10 days (see Figure 12).

Visual quality shelf-life:

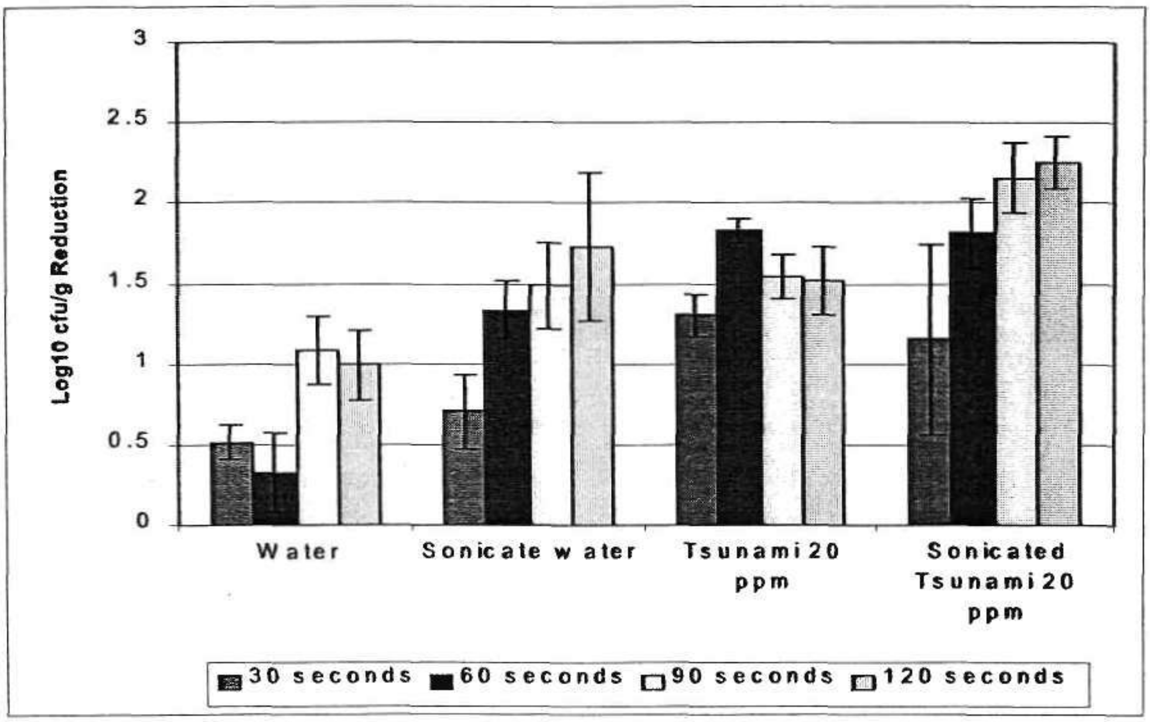
Deterioration in quality of the shredded lettuce was observed during storage for 7 days at 4°C. The ultrasonic washed shredded lettuce became flaccid and softened during storage, and was unacceptable after 7 days, compared with the chilled washed product that remained crisp and in good condition.
The chilled washed broccoli florets became dry and developed moderate whitening on the cut stalk, despite this disorder the product was considered to be of good quality after 10 days of storage. The ultrasonic washed broccoli florets remained moist, as consequence no whitening occurred. The product was of very good quality at 3 days, however by 7 days the moisture increased was unacceptable and by 10 days an off-odour had developed. The chilled washed carrot slices became dry and developed moderate to severe whitening during storage, at 10 days the product was unacceptable. The ultrasonic washed carrot slices remained slightly moist, and no whitening was observed at 10 days the product was in very good quality.

Figure 1. Effect of water temperature on the microbial reduction during non-sanitised and sanitised ultrasonic washing of shredded lettuce



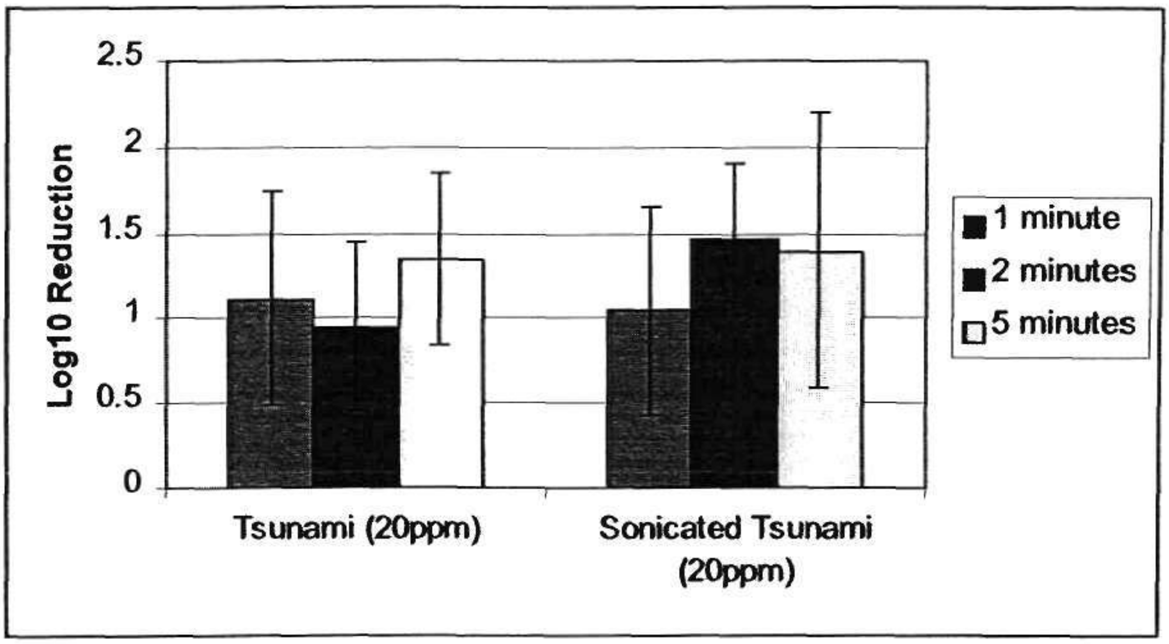
Note: Tsunami concentration at 20 ppm peracetic acid

Figure 2. Effect of contact time on microbial reduction during heated non-sanitised and sanitised ultrasonic washing of shredded lettuce



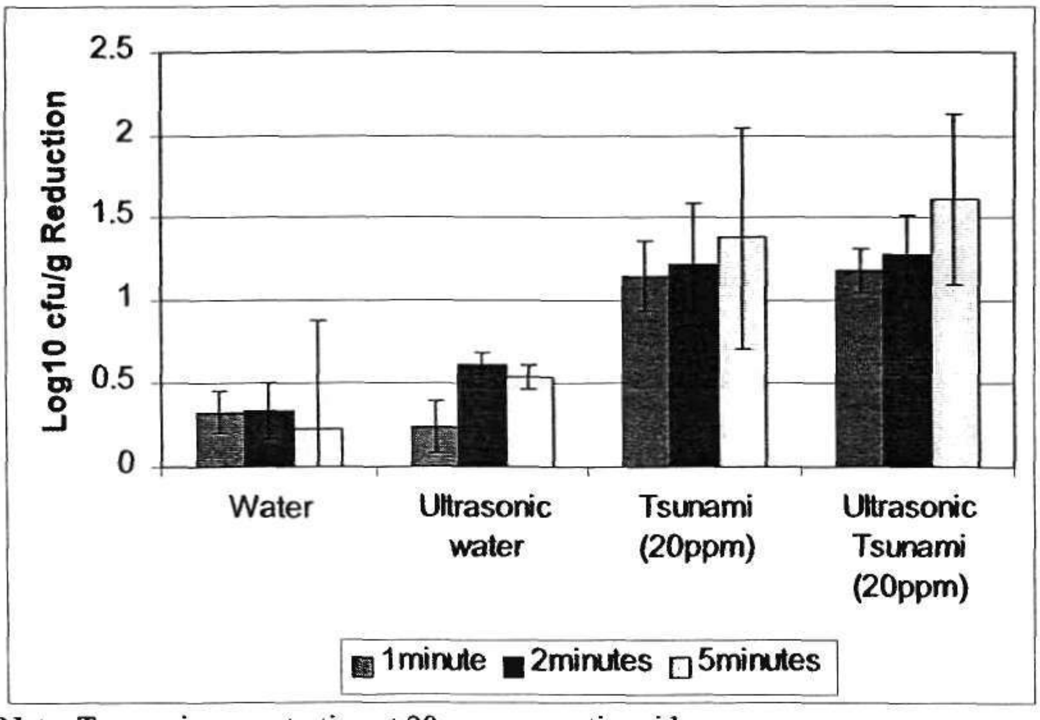
Note: Tsunami concentration at 20 ppm peracetic acid

Figure 3. Effect of contact time on the microbial reduction during heated (50°C) ultrasonic washing of broccoli florets



Note: Tsunami concentration at 20 ppm peracetic acid

Figure 4. Effect of contact time on the microbial reduction during chilled (50°C) ultrasonic washing of carrot batons



Note: Tsunami concentration at 20 ppm peracetic acid

Figure 5. Effect of ultrasonic operating functions on the microbial reduction during the washing of shredded lettuce

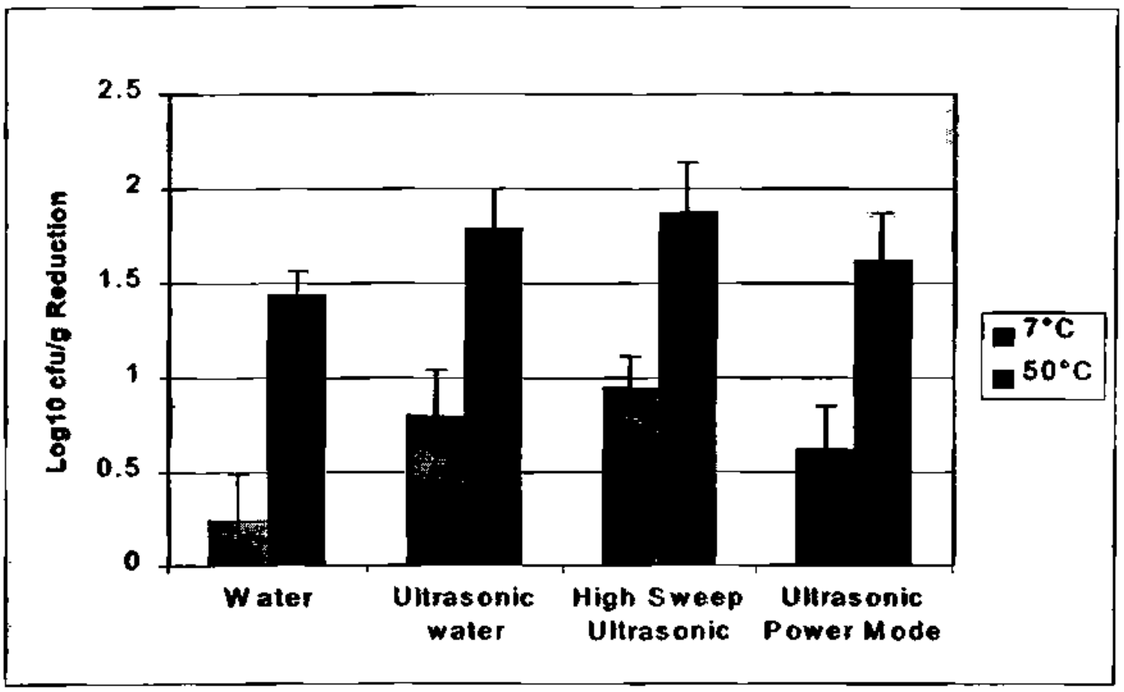
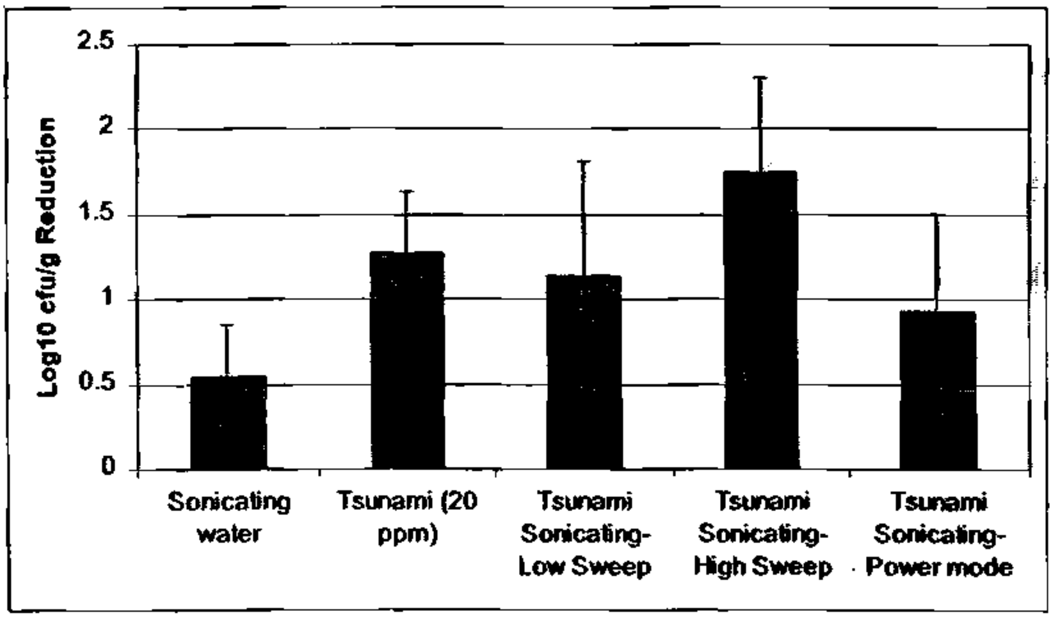


Figure 6. Effect of ultrasonic operating functions on the microbial reduction during chilled (4-5°C) sanitised washing of shredded lettuce



Note: Tsunami concentration at 20 ppm peracetic acid

Figure 7. Effect of increased organic load on the microbial reduction during heated (50°C) sanitised ultrasonic washing of shredded lettuce

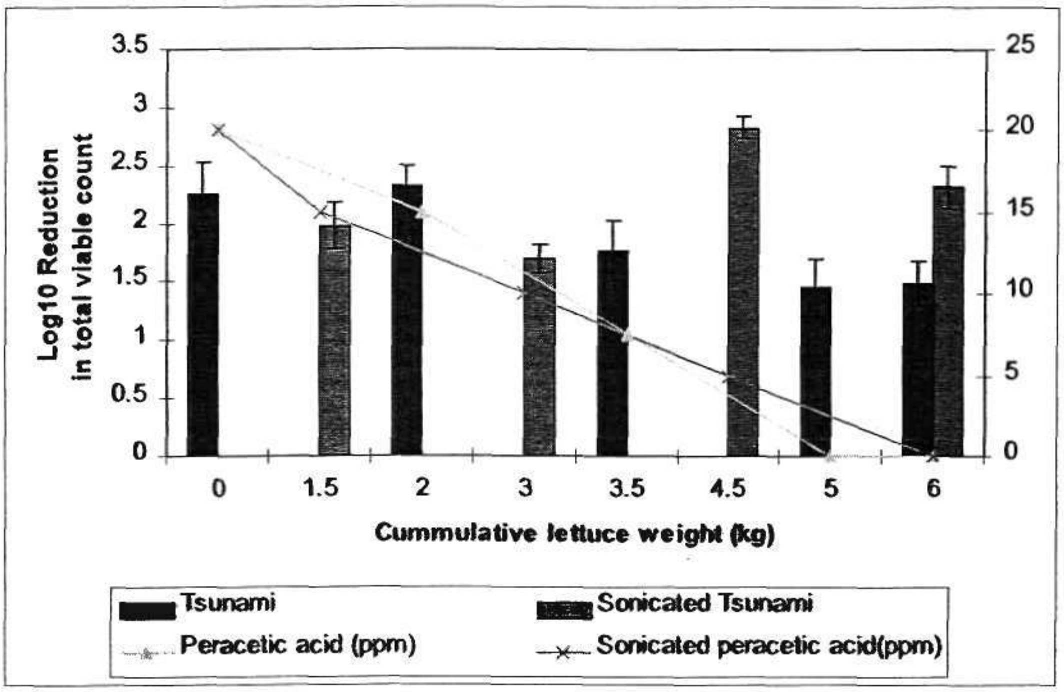


Figure 8. Effect of increased organic load on the microbial count in the washing water during heated (50°C) sanitised ultrasonic washing of shredded lettuce

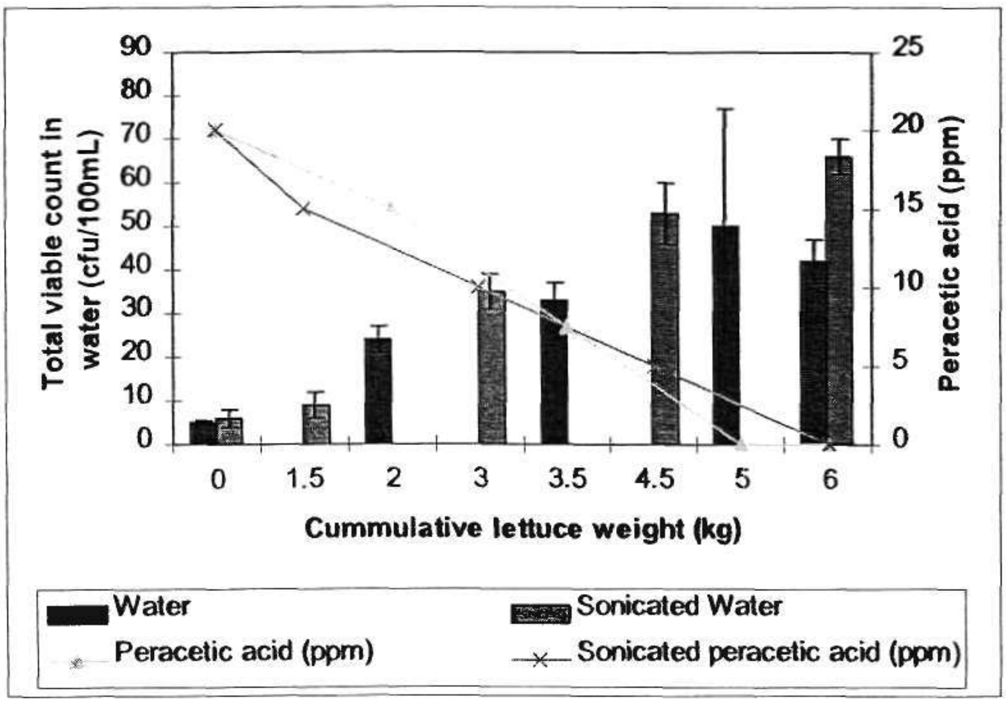
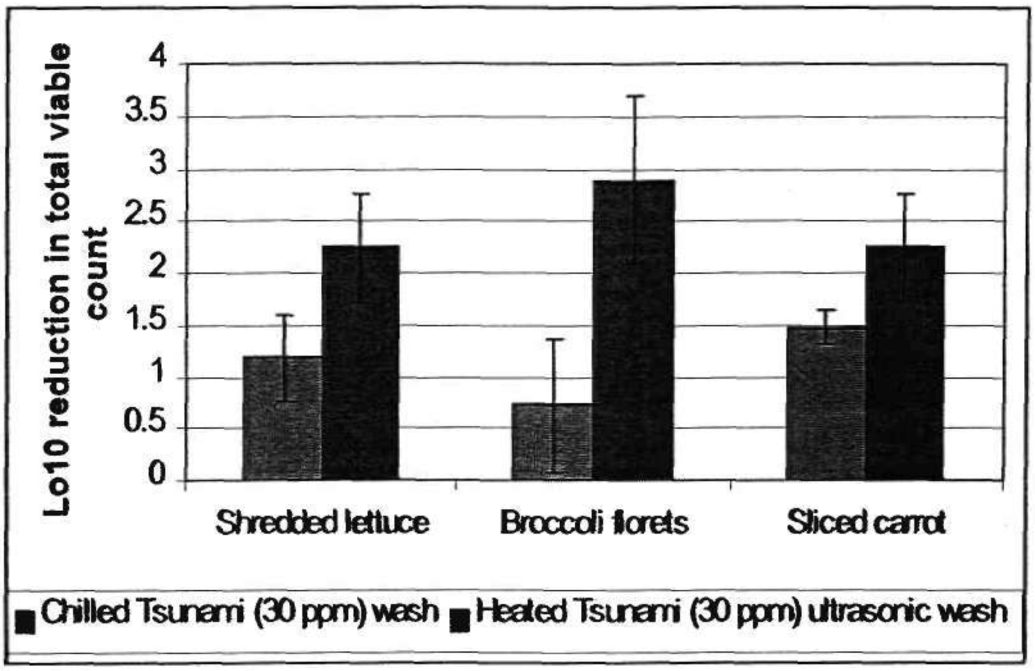


Figure 9. Comparison in the microbial reduction between industry practice of chilled (4°C) sanitised washing and sanitised heated (50°C) ultrasonic washing for shredded lettuce, broccoli florets and carrot slices.



Note: Tsunami concentration at 20 ppm peracetic acid. Two stage washing process; shredded lettuce washed for 90 seconds, broccoli and carrots washed for 5 minutes then followed by rinse for 1 minute in chilled water (4°C).

Figure 10. Total viable counts on shredded lettuce during storage at 4°C.

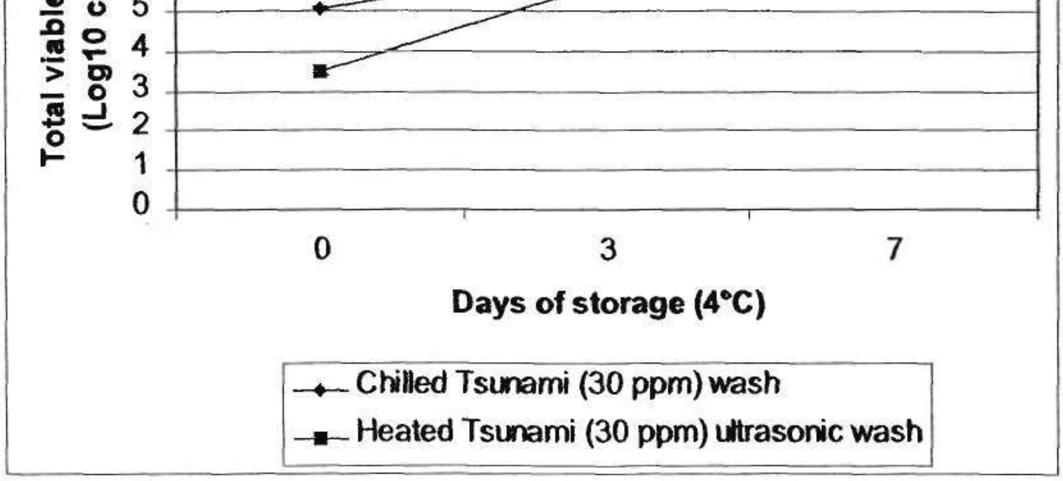


Figure 11. Total viable counts on broccoli florets during storage at 4°C.

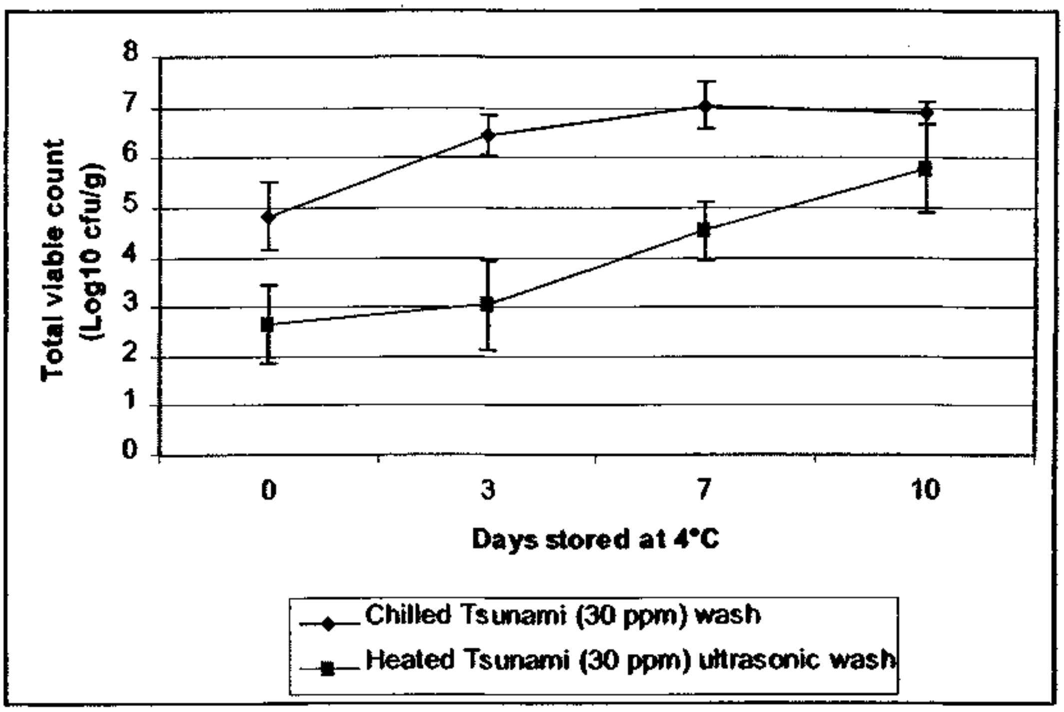
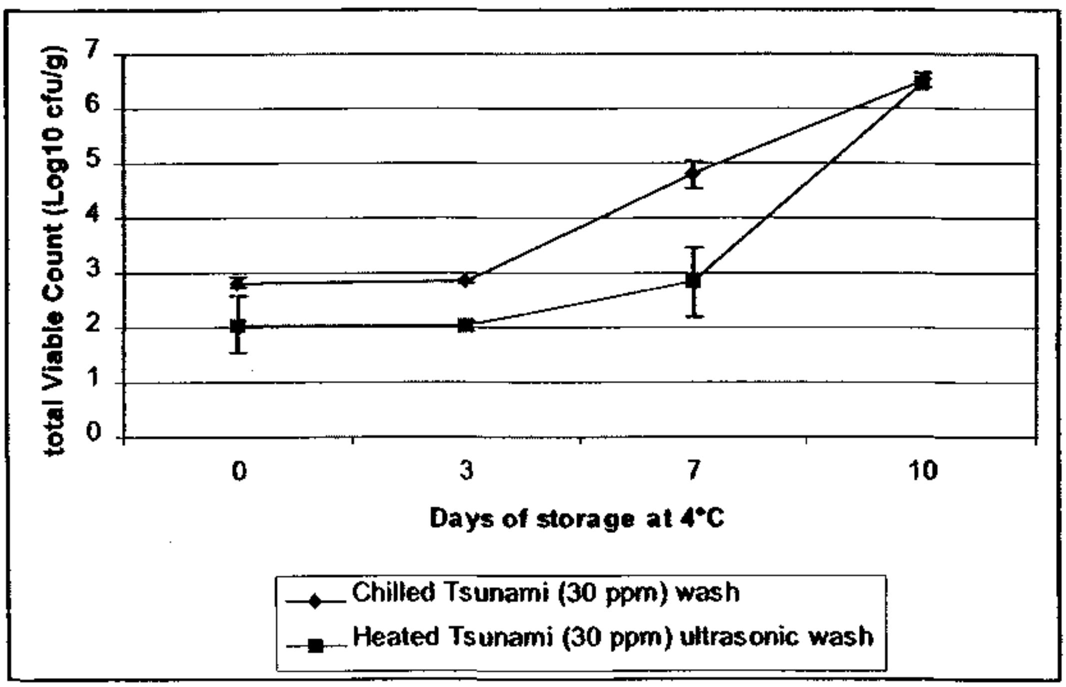


Figure 12. Total viable counts on carrot slices during storage at 4°C.



DISCUSSION

The washing of minimally processed vegetables generally involves the use of chilled sanitised water (2°C to 8°C). Chilling of the washing water is performed to retain product quality by reducing the potential for microbiological growth, enzyme activity and product respiration during processing.

The ultrasonic washing was not effective when used in chilled water (4-5°C). Cavitation was probably not adequately achieved in the chilled washing water, the slight improvement observed in the reduction of the average total viable count for both sanitised and non-sanitised washing was not operationally significant (as established from industry consultation).

Washing of the lettuce at 45-50°C has been reported to reduce the browning on shredded lettuce (Loaiza-Verlarde, J.G., 1997 and Delaquis, P.J., et al., 1999). As this approach provided an alternative treatment for the washing of minimally processed vegetables and a more suitable temperature to apply ultrasonic washing, trials were conducted at 45-50°C. When compared with the industry practice of chilled washing, application of sanitised ultrasonic washing at 50°C was more effective in reducing the total viable population on shredded lettuce, broccoli florets and carrot slices (see Figure 9). The carrot batons were pre-washed during abrasive peeling prior to the sanitised ultrasonic washing trial, consequently the microbial reduction was generally low. The reduction in the total viable population was due to the combined effect of heated washing water, sanitation and ultrasonics. Each individual treatment also was demonstrated to provide a greater reduction in the total viable populations (see Figure 1). These findings are comparable to the additional 1 Log cfu/g reduction reported with ultrasonic washing when using 100 ppm chlorinated water from Campden & Chorleywood Food Research Association (personal communication, Dr Ian Seymour). These findings were conducted on a smaller 2 litre system (15-20 litres used in this project), which is presently being scaled up to a 40 litre system.

The quality of the lettuce washed in sanitised heated ultrasonic water was inferior to the sanitised chilled washed lettuce. This was partially an effect of ultrasonic washing time and washing temperature. A shorter ultrasonic washing period of 1 minute or less reduced the product damage however did not provide improved reduction in the total viable count (see Figure 2). The deterioration in product quality from the effect of heated ultrasonics was avoided at 35°C, however this temperature was not effective in reducing the enzymatic browning. At 35°C the application of ultrasonics provided an increased reduction in the total viable number by 0.64 Log cfu/g. An ultrasonic washing application of 40°C for 1 to 1.5 minutes is likely to provide the greatest total viable count reduction while still retaining good product quality.

The visual quality of the broccoli florets was very good for 3 days of storage, but rapidly deteriorated to be unacceptable after 7 days at 4°C. In consideration of the results with shredded lettuce, the application of ultrasonics on broccoli at a lower temperature (40°C) for a shorter treatment time should be beneficial for product quality, but this was not evaluated.

The visual and microbiological quality of the carrot slices were both improved with the sanitised-heated ultrasonic washing. The application of high temperature and acid treatment has been previously demonstrated to inhibit the development of whitening on carrot surfaces (Bolin, H.R. and Huxsoll, C.C., 1991). Ultrasonic washing may be applicable for stronger textured vegetable products such as potatoes and pumpkins.

With an increasing organic load, a similar rate of decline in peracetic acid concentration was observed with and without ultrasonic application (see Figure 7). When the peracetic acid had been completely declined, the average reduction in total viable population was higher for ultrasonic washing (see Figure 7). The peracetic acid breaks down to acetic acid carbon dioxide and water. The presence of the acetic acid maintained a reduction in the total viable population that was greater then that normally achieved with water. In addition, the acidified water would have also assisted in limiting the total count in the washing water to below 100 colony forming units per 100mL.

CONCLUSIONS

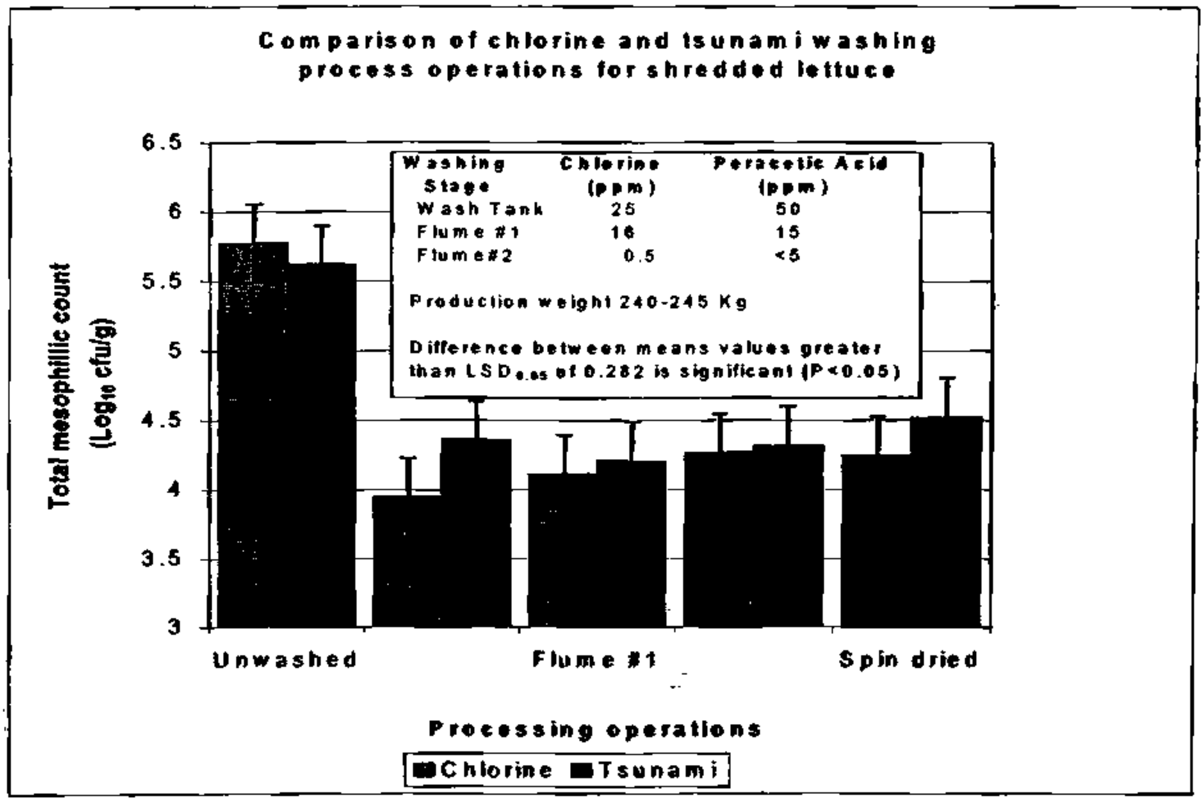
Ultrasonic washing of minimally processed vegetables is not suited to the general chilled washing applications currently conducted by growers and fresh-cut processors. Ultrasonics could be applicable to situations were the product is undertaking a mild heat treatment to facilitate cavitation and where the application of sanitisers was to be avoided. The treatment of carrot slices was identified as a suitable product suitable for heated ultrasonic washing with peracetic acid.

References

Bolin, H.R. and Huxsoll, C.C. (1991). Control of minimally processed carrot surface discolouration caused by abrasive peeling. J. Food Sci. 56: 416-418.

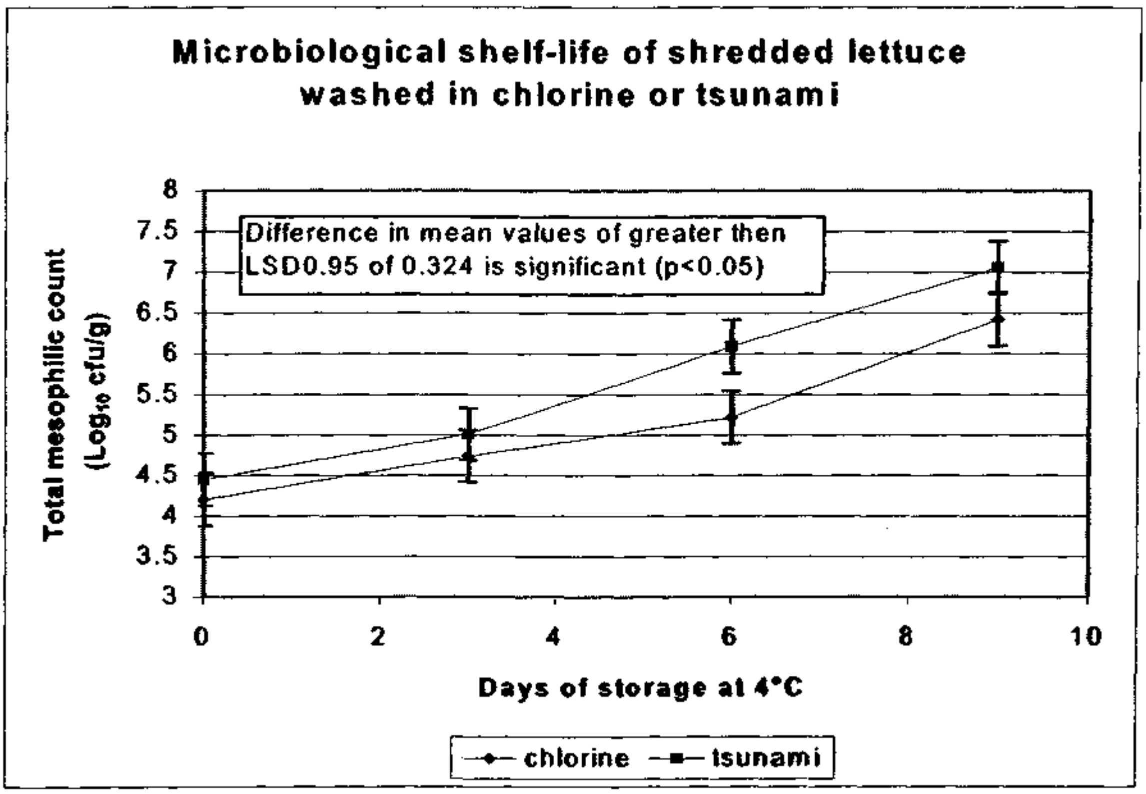
Delaquis, P.J., Stewart, S., Toivonen, A.L. and Moyls, A.L. (1999) Effect of warm chlorinated water on the microbial flora of shredded iceberg lettuce. Food Research International 32: 7-14.

Loaiza-Verlarde, J.G. (1997) Effect of intensity and duration of heat-shock treatments on wound-induced phenolic metabolism in iceberg lettuce. Journal of American Society for Horticulture Science 122(6): 873-877.



The shredded lettuce was vacuum packaged in 2.5 kg bags (PD???, Cryovac) then stored at 4°C. The chlorine and Tsunami washed products retained a very good appearance after 9 days of storage, without any browning and a slight loss in crispness. The package atmosphere for both products equilibrated at a range of 0.2-1.5 % oxygen and 15-18% carbon dioxide. Immeadiately after washing and at 3 days of storage there was no significant difference in the total mesophillic counts. However, following 3 days of storage the total mesophillic population grew faster on the Tsuanmi treated product attaining significantly higher counts then the chlorine washed product on days 6 and 9.

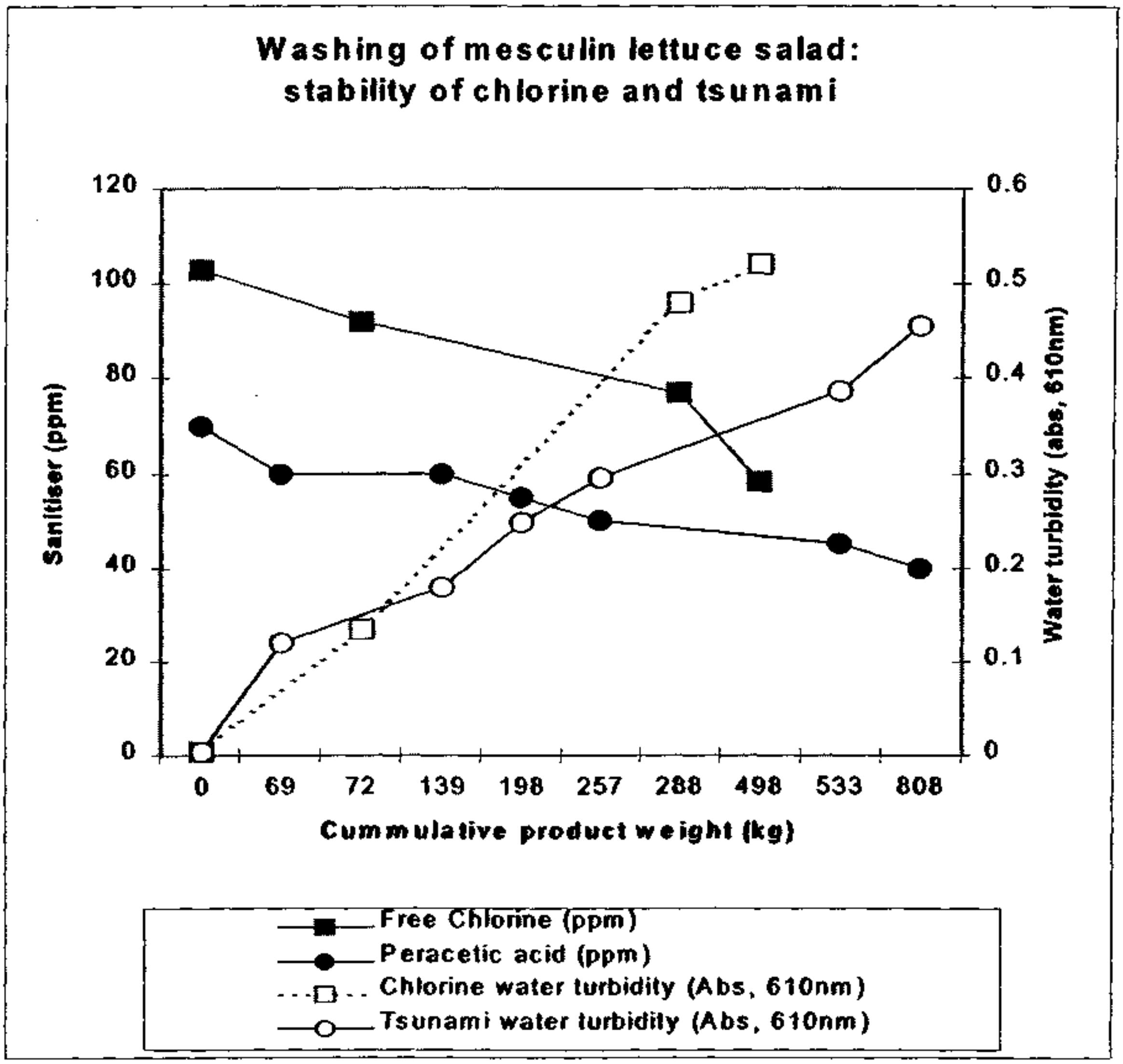
Figure 4:



Comparison of washing mesculin lettuce salad with chlorine or Tsunami treated water:

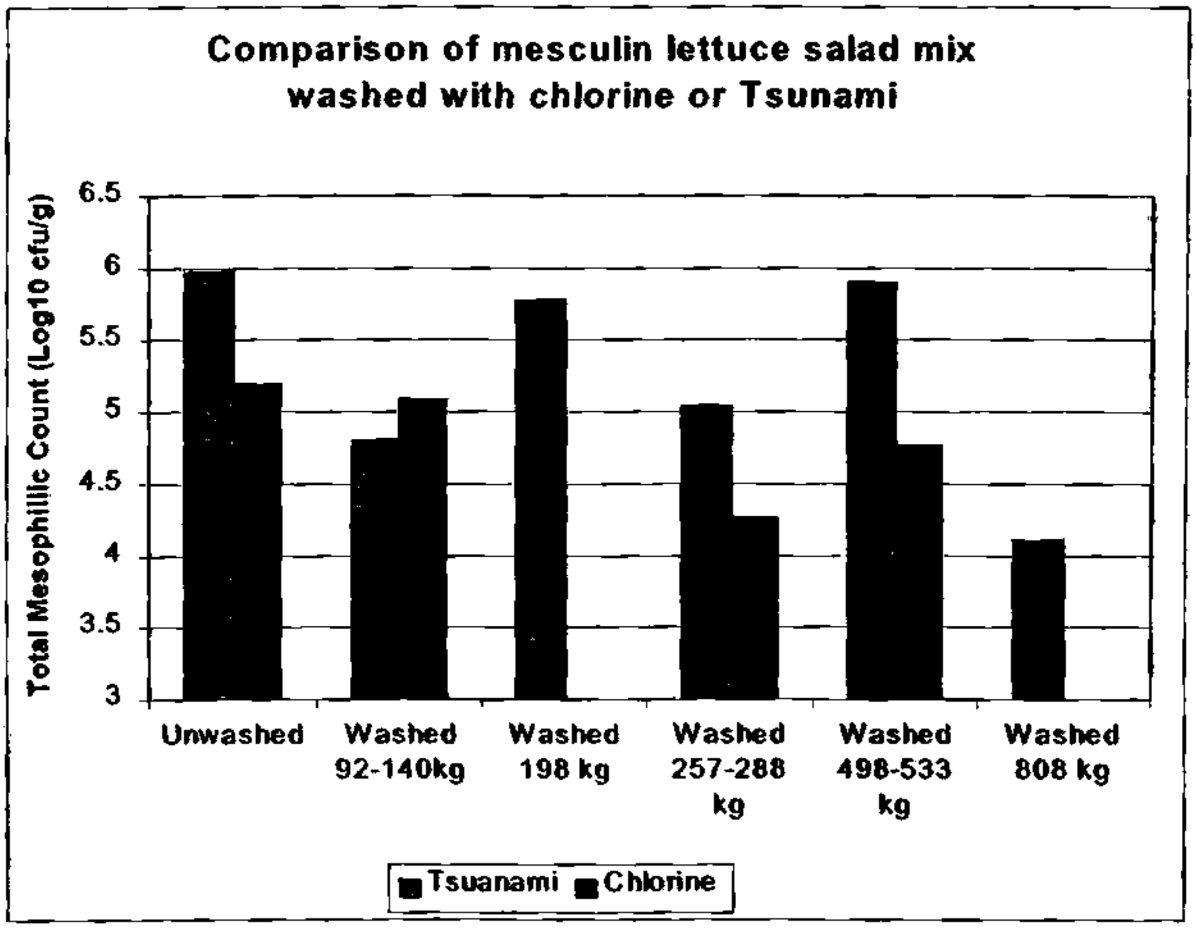
The initial level of free chlorine at 103 ppm rapidly declined throughout the processing shift to 60 ppm, following the production of about 500 kg of lettuce. Throughout this period the pH remained between 5.63-6.03. In comparison with Tsunami the peracetic acid commenced at 70 ppm and decline at a slower rate to 40 ppm, following the production of about 800 kg of lettuce (see Figure 5). This corresponded to a % decline of chlorine and Tsunami sanitiser of 43% and 35% respectively, based on 500kg of washed mesculin lettuce salad. The organic load in both washing systems was measured using ATP-bioluminescence. The levels were less then 1000 RLU, which was comparatively much lower then levels in excess of 1,000,000 for shredded lettuce. Less organic material is released from the mesculin lettuce because there is only a single cut on the main stem. The mesculin lettuce leaves have a high soil loading, therefore turbidity (Abs, 610nm) was used as an indicator of water quality. The turbidity of the washing increased rapidly for both washing systems during the processing shift (see Figure 5). The high organic load released from the shredded lettuce caused a more rapid depletion of both the sanitisers then the high soil loading present when washing the mesculin lettuce salad. No acetic acid (a by-product of peracetic acid) or hydrogen peroxide gas was detected above the washing tank

Figure 5



The microbial reduction achieved with both chlorine and Tsunami washing of mesculin lettuce salad was sparodic. In some instances during the production shift the microbial reduction was minimal where as at other times a 1-2 log reduction was achieved. There was a large variability in product counts at each sampling period. This is likely to be a consequence of the non-uniformity of the product, which consisted of between 9-13 different lettuce types. Therefore it was impossible to conclusively establish a comparison between the sanitisers (see figure 6).

Figure 6



There was a carry over of chlorine on the product into the 1st and 2nd water rinsing flumes, attaining free chlorine levels of 30 ppm and 6 ppm respectively. The carry over of chlorine in small quantities was favourable for maintaining the microbiological quality of the product (see Figure 7) . The total mesophillic count in the 1st and 2nd water rinsing flumes remained low (see Table 2), despite the soil loading increasing in water turbidity (measured by Abs, 610nm) to an average of 0.361 and 0.202. There was also a carry over of Tsunami into the 1st and 2nd water rinsing flumes, attaining peracetic acid levels of 15 ppm and <5 ppm respectively (see Figure 3). The total mesophillic counts in the Tsunami treated washing water were considerably higher (88-292 cfu/100mL), then for the chlorinated washing system (see Table 2).

Table 2: Microbiological quality of washing water for mesculin lettuce salad

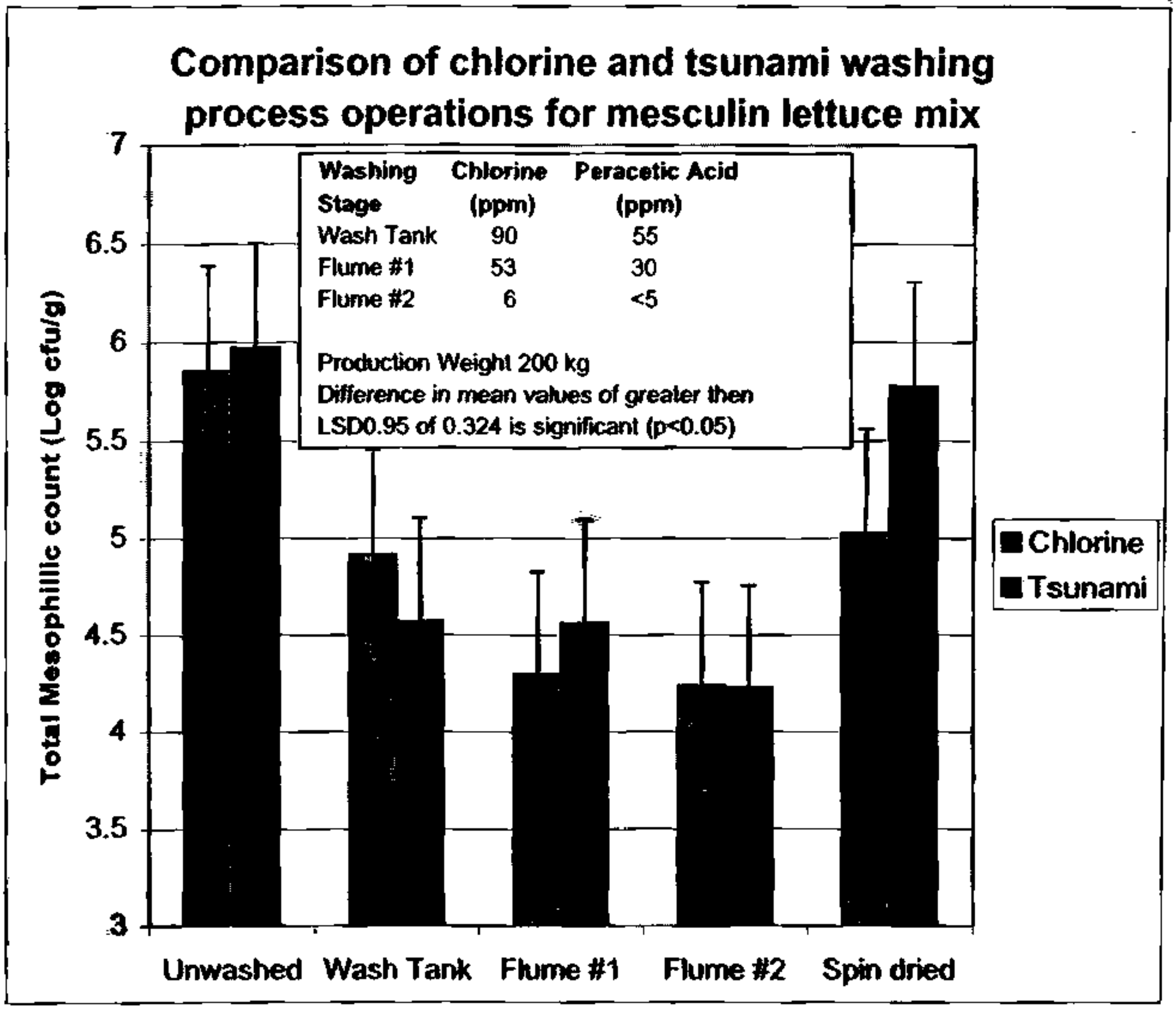
Processing operation	Chlorine washing	Tsunami washing

(shredded lettuce production weight in kg)	**water (cfu/100mL)[1]**	**water (cfu/100mL)[1]**
Wash tank (pre-wash 0 kg)	<1	245
Wash tank (69-72kg)	1	7,600
Wash tank (257-288 kg)	1	11,600
Wash tank (498-533 kg)	1	31,000
Flume #1 (240-245 kg)	<1	18,500
Flume #2 (240-245 kg)	2	16,800

[1]Average, n=2

There was a significant difference between the unwashed and product samples collected from the wask tank and the 2 rinsing flumes for both the chlorine and Tsunami washing systems (see Figure 7). Flume rinsing appeared beneficial for the microbiological quality of the product for both the chlorine and Tsunami washing systems, reducing the average total mesophilic population. A significant decrease in the microbiological count on the chlorine washed product occurred following flume rinsing. The total microbiological count for the spin dried product was on average higher then product sampled from the washing tank or flumes. This maybe due to the residual effect of sanitiser on the product with samples taken directly from the washing tank or flumes. There was a large variability in washed product (sampled after spin drying) throughout the processing shift (see figure 6). Increased variability due to the non-uniformity of the product (9-13 different lettuce types) has made it difficult to conclusively establish a comparison between the process operations.

Figure 7:



Shelf-life was not completed for mesculin shredded lettuce.

DISCUSSION

Tsunami was a more stable sanitiser then chlorine when there was a high organic and soil load. The microbiological reduction achieved by both sanitisers was not significantly different. There was no chlorine, acetic acid or hydrogen peroxide gases detected directly above the washing tank. Therefore using this style of washing system there was no occupational health and safety concern in relation to sanitiser and by-product gas-off. The product in the washing tank is mixed using large water jet manifold system with minimal aeration. Evaluation is also required for a system that uses air jets or creates aeration at the water surface that may result in sanitiser gas-off.

A larger proportion of both sanitisers was transferred into the rinsing flumes with the mesculin lettuce salad compared with the iceberg salad. Due to the larger size and shape of the mesuclin lettuce they trap more of the sanitised washing water which was transferred to the rinsing flume.

Mesculin flume rinse had a microbial reduction trend probably due to large level of sanitiser carry over compared with the shredded lettuce product.

The appearance of shredded iceberg lettuce following chlorine or Tsunami washing and storage at 4°C for 9 days was very good, without browning, but with a slight loss in crispness. The controlling of browning is also dependent on using permeable packaging to attain a modified atmosphere. The microbiological counts on the Tsunami washed product was significantly higher at the end of shelf-life.

CONCLUSIONS

- **Tsunami and chlorine provided similar product sanitation**

- **Tsunami was more stable then chlorine during high organic washing load.**

FURTHER WORK

- **Shelf-life of mesculin lettuce salad**

- **On-line monitoring and dosing control for Tsunami**

Milestone #9: Preliminary investigation of ultrasonic washing on minimally processed vegetables

EXECUTIVE SUMMARY

Preliminary trials were conducted to evaluate the suitability of ultra-sonicated washing on shredded lettuce based upon microbiological efficacy. Small scale trials were conducted in a 20 litre sonication tank with batches of shredded lettuce. Washing shredded lettuce in sonicated water achieved a significantly ($p<0.05$) greater reduction in the total mesophillic count on the shredded lettuce, compared with washing in water without sonication. The use of sonicated water achieved a total microbial reduction in the range of 0.61 to 1.26 Log on shredded lettuce for a period of 2 to 5 minutes. This was increased to a 1.7 Log reduction on average for an extended sonication period of 12 minutes. However, the longer period of application is not practical for commercial application and resulted in visible damage to the lettuce surface. Further trials combining sanitisers with sonication were conducted for 2 minute periods. Batch washing trials demonstrated that the reduction in the total mesophillic count on the shredded lettuce was significantly ($p<0.05$) greater using sonicated chlorine or Tsunami treated water compared with the non-sonicated application of both saintisers. A continuous washing system was operated by recycling the sonicated washing water through a heat exchanger to maintain the water temperature at 3-4°C. The washing water in the sonication tank was replaced approximately every 5 minutes. Using this system several batches of shredded lettuce were washed in sequence. The organic level in the washing water rapidly increased (as measured by total ATP levels) with the consequence of a complete reduction in the effect of sonication. The sonication system was incompatible for washing water with a high organic load . Further trials will be conducted with a more advanced sonication system which is designed to adapt for varying loads in the sonication washing tank. Scalime have made an expression of interest in the continuance of this research because it represents a potential physical alternative to chlorine washing, or could be used in combination to enhace existing systems or reduce the required levels of chlorine. Further preliminary trials are required to provide sufficient performance data to justify the design and construction of a pilot-scale system suited for fresh-cut operations.

INTRODUCTION

Ultrasonication can create ultrasound waves (UW) generated by mechanical vibration frequencies higher than 15kHz. When these waves are propagated in a liquid they produce alternating compression and expansion cycles. During the expansion cycle, UW make small air bubbles grow in a liquid. When they reach a volume at which they can no longer absorb more energy, they implode violently. This phenomenon is known as cavitation. During implosion, very high temperatures and pressures are reached inside the bubbles. This effect is thought to be the main reason for the bactericidal effect.

The effect of a santising agent (ie. chlorine) is less effective on bacteria that are attached and trapped on the product surface (ie.formation of biofilms). It has long been established that UW can break up bacterial clumps of bacteria that naturally occur in milk (stone and Fryer, 1984), including the removal of bacteria from milk bio-films on metal surfaces (Daufin and Saincliviert, 1967). UW was demonstrated to improve surface sterilisation of poultry skin in combination with chlorine (Lillard, 1994, Sams and Feria, 1991).

The application to fresh-cuts would utilise ultrasonic cleaning systems normally used for cleaning equipment. This requires a power supply for generating ultrasonic energy and immersible transducers which are mounted in the washing tank to optimise the distribution of UW.

PROJECT AIM

Evaluate the suitability of using ultra-sonication for the washing of fresh-cut vegetables, based upon microbiological and organoleptic quality.

METHODS

Description of ultrasonication washing system:

An ultrasonication system was leased from Ultrasonics Consolidated (P/L) with the following specifications.
Volume: 15 litres
Frequency output:40 MHz

Temperature control set up: 3-4°C
Total volume: 40 litres (including heat exchanger)
Flow rate: 300 litres per hour

Product description:

Shredded iceberg lettuce of about 1cm in width.

Microbiology

Products were sampled in triplicate for microbiological analysis, 20 gram portions were homogenised (Colworth Stomacher) in 180 mL of 0.1 % bacteriological peptone (Oxoid), then spiral plated in duplicate on plate count agar (Oxoid) and incubated for 2 days at 30°C.

Water samples were filtered through TTC filter pad (Sartorious), then placed on a moistened nutrient pad and incubated for 2 days at 30°C.

Package headspace gas analysis

Gas analysis of the packaged headspace for carbon dioxide and oxygen content. Packaged headspace was analysed using a MAPtest 4000 equipped with an infra-red carbon dioxide analyser and zarconia sensor for oxygen

Statistical Analysis

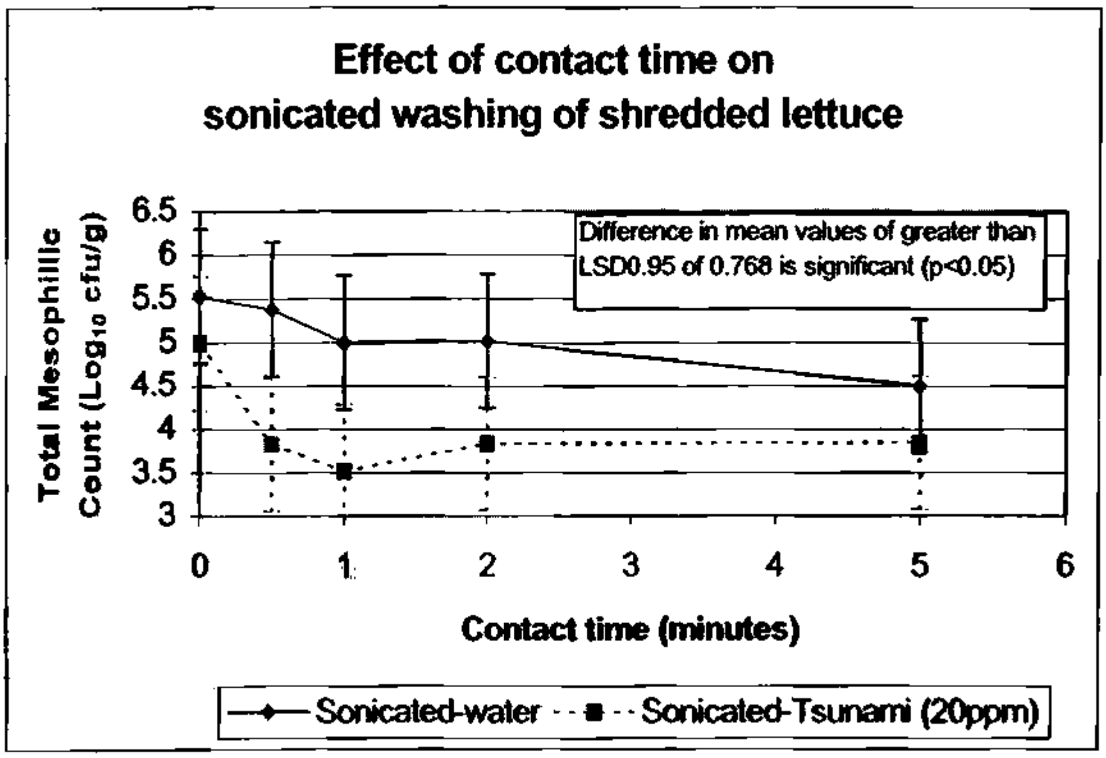
The mean of 3 replicate determinations are presented with respective standard deviation. Analysis of variance (single factor) was calculated using Genstat version 3.2 for calculation of least significant difference ($LSD_{0.95}$). Difference between mean values greater then $LSD_{0.95}$ is significant, at $p<0.05$.

RESULTS

Effect of contact time on sonicated washing

Washing the shredded lettuce in sonicated water for 30 seconds provided a marginal reduction in the total mesophillic count on the shredded lettuce. Approaximatley 0.5 log reduction was achieved with 1 to 2 minutes sonicated washing time. Greater than a 1 log reduction was achieved with a treatment of 5 minutes (see Figure 1). Prolonged treatment for 12 minutes enabled a microbiological reduction of 1.5 Log, but the lettuce tissue was visibly damaged, becoming translucent (see Figure 2).

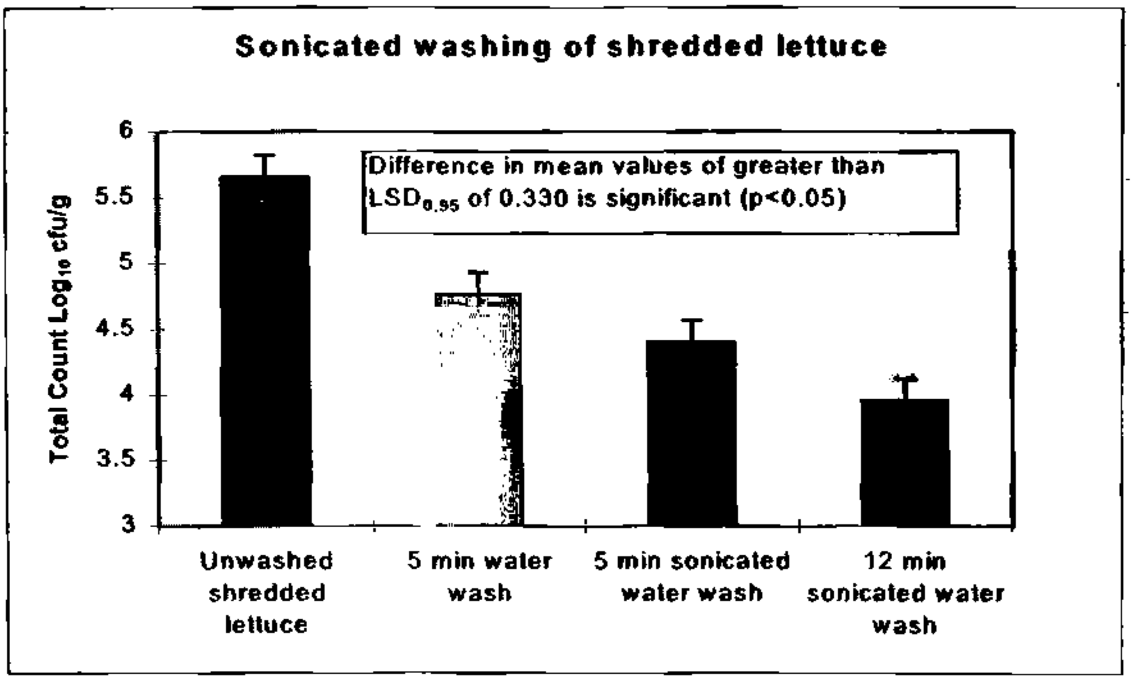
Figure 1.



The total mesophillic count on the shredded lettuce washed in sonicated water was significantly lower than that washed in water without sonication (see Figure 2).

Investigation of the effect of contact time when washing shredded lettuce in sanitised-sonicated water demonstrated that a significant reduction in the total mesophillic count was achieved with a contact time of only 30 seconds. There was no additional benefit with extending the contact time beyond 1 minute (see Figure 1).

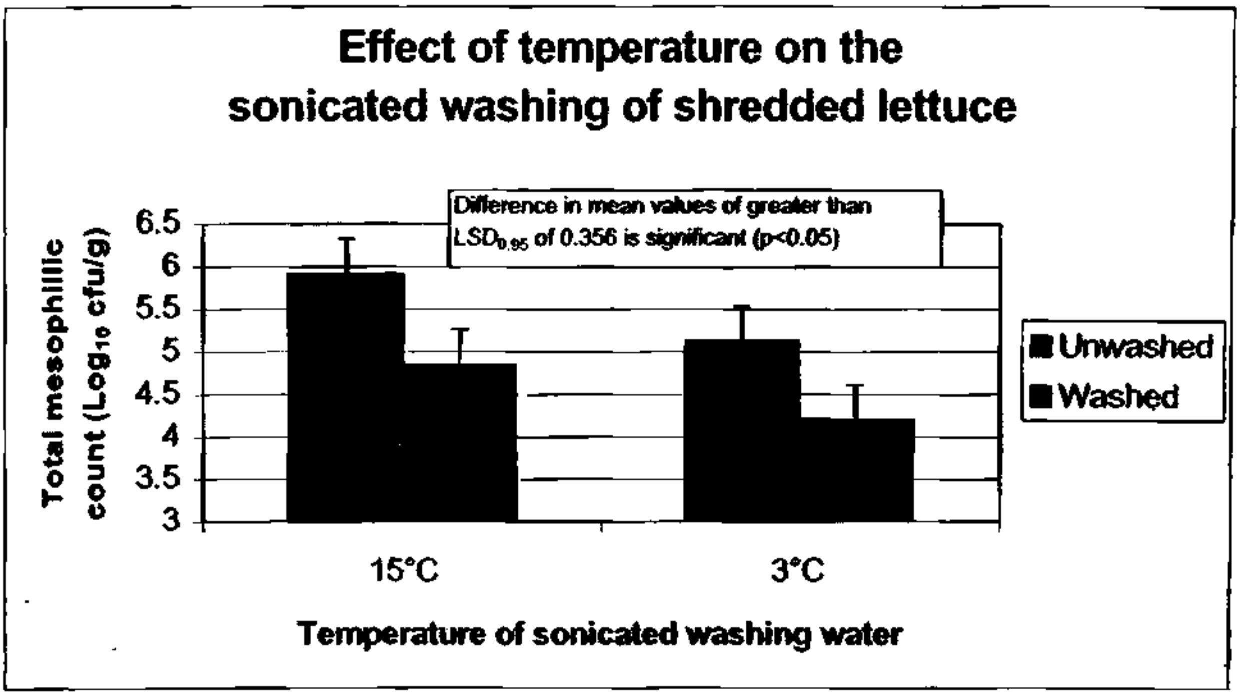
Figure 2:



Effect of temperature on sonicated washing

The sonicated washing water was cooled by recycling it through a heat exchanger and returning it to the washing bath. The reduction in total mesophillic count on the shredded lettuce was comparatively similar at both 15°C and 3°C, achieving 91% and 87% respectively (see Figure 3).

Figure 3:



Sanitised sonication washing system

Washing shredded lettuce in sonicating water achieved a significant reduction in total mesophillic count compared with water washing (see Figure 4). A sonicating water wash was comparable with washing the shredded lettuce in Tsunami treated water at 10 ppm peracetic acid. Sonicating the Tsunami treated washing water achieved a significant improvement in the reduction of the total mesophillic count compared with water washing (see Figure 4). The sanitation performance of chlorine was also significantly improved by sonicating during the washing of shredded lettuce (see Figure 5).

Figure 4:

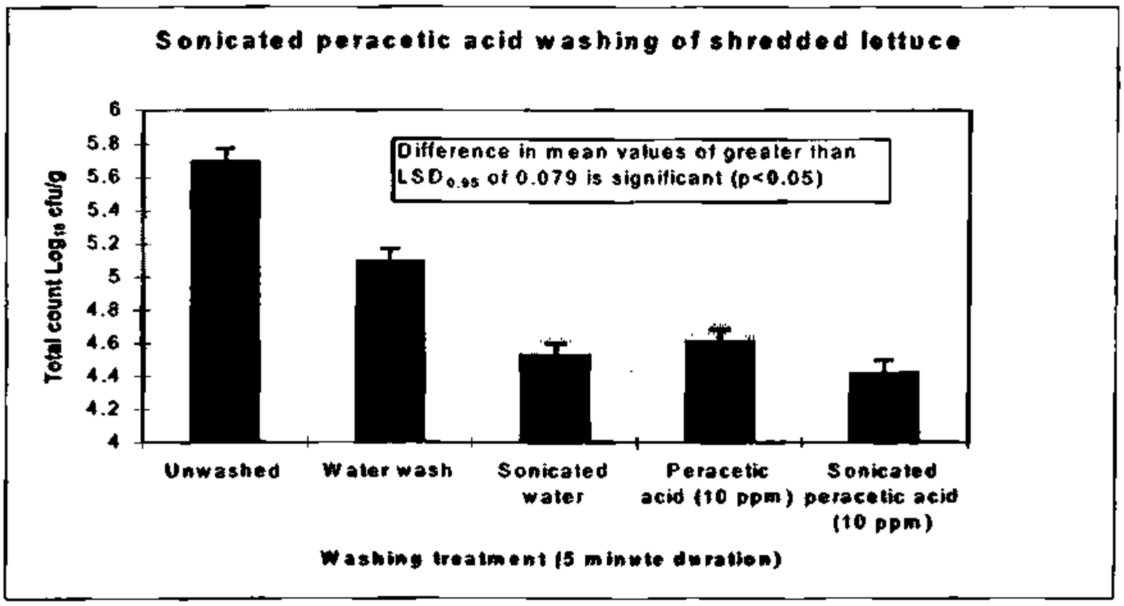
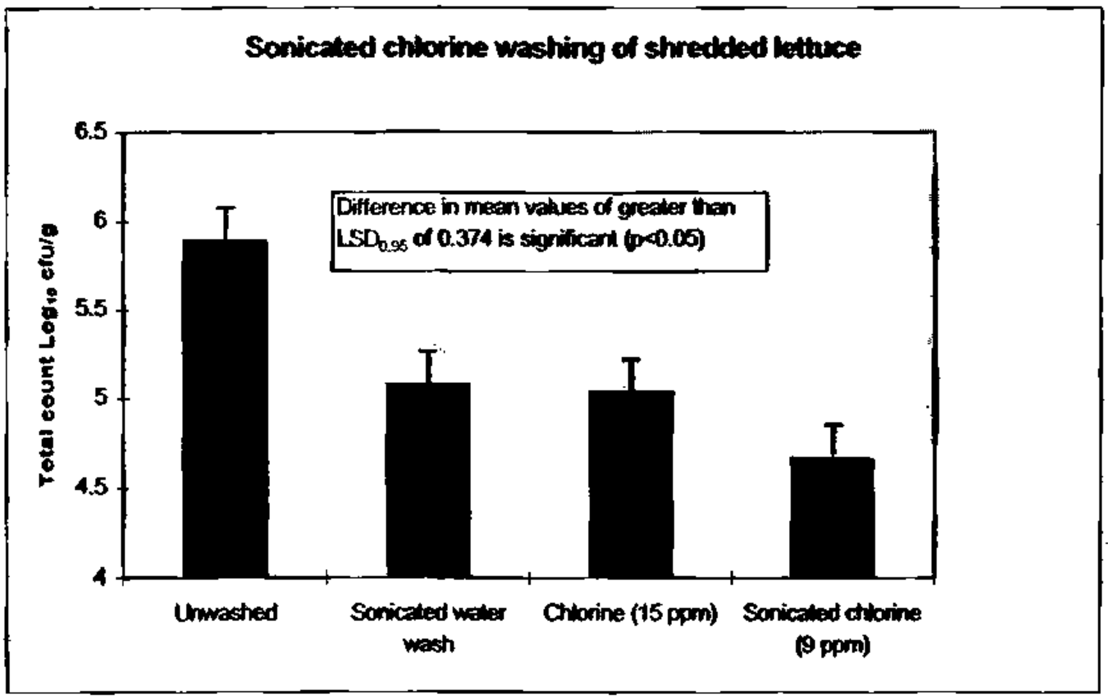


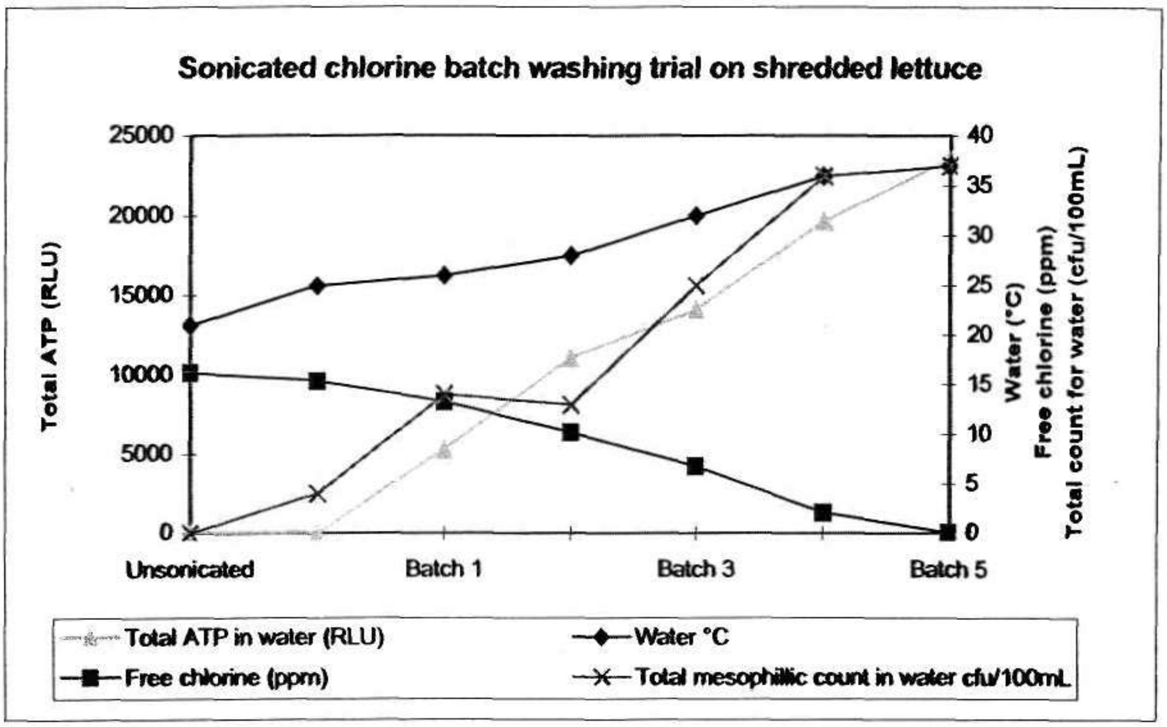
Figure 5



Batch washing trial

A batch washing trial was conducted with sonicating chlorinated washing water. During sonication the temperature increased from 25°C to 37°C (see Figure 6). Without water recycling or screening the organic load increased in the sonication bath with the addition of each batch of lettuce. The total mesophillic count in the washing water increased to 37 cfu100mL. The chlorine levels were rapidly reduced to less then 1 ppm, due to gass-off with the raised water temperature (see Figure 6). Consequently, water recycling and temperature control is required to reduce sanitiser gass-off, microbial growth and product respiration rate.

Figure 6:

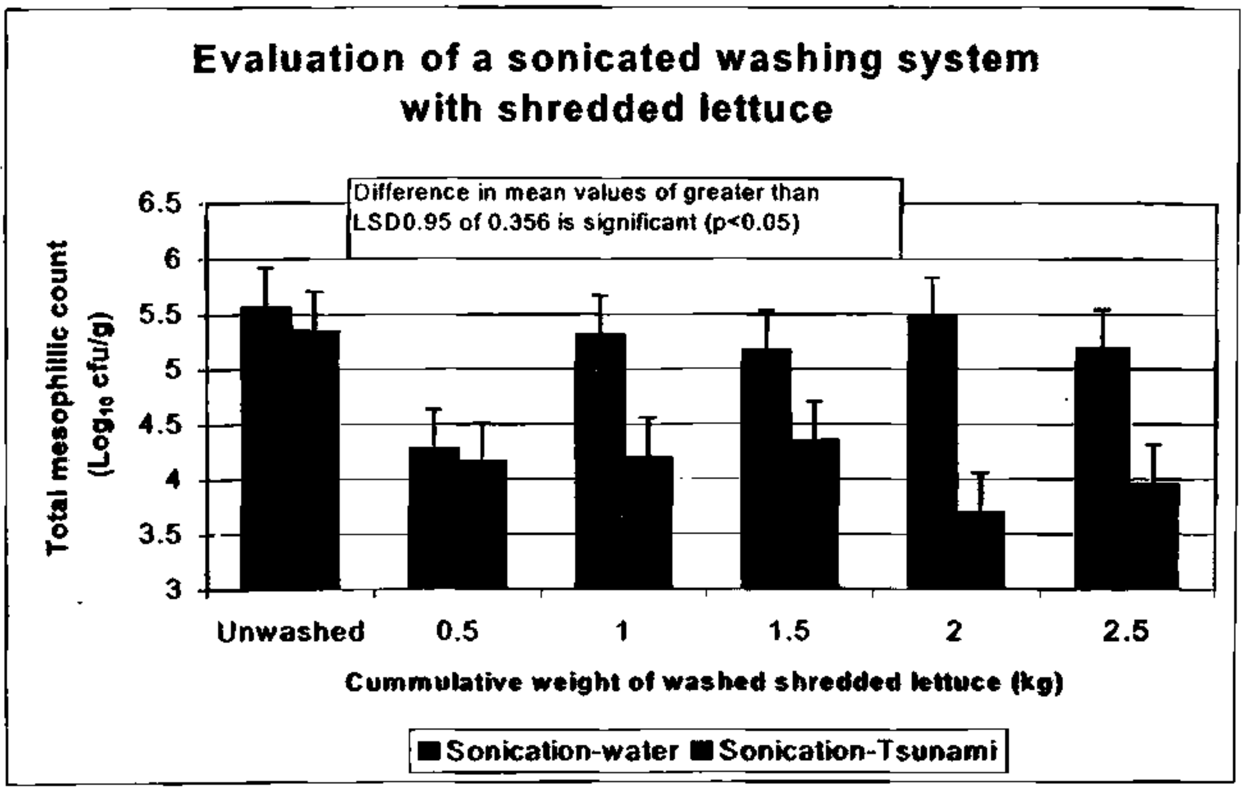


Water recycling and temperature control

The temperature of the water in the sonicating washing bath (15 litres) was held between 2.9-3.7°C by pumping the water through a heat exchanger. The total volume of the washing bath was recycled approximately every 5 minutes.

When washing the first batch of shredded lettuce in the sonicating water bath a significant reduction in the total mesophillic count was achieved. For the subsequent batches of shredded lettuce there was no significant reduction in the total mesophillic count. The ineffectiveness of the sonication with subsequent batches maybe due to the effect of water recycling reducing the gassing-off and the accumulation of the organic load. When using Tsunami (20 ppm) treated water in the sonication bath a significant reduction in the total mesophillic count was achieved throughout the trial (see Figure 7). The concentration of peracetic acid was maintained during the washing trial at 20 ppm.

Figure 7:



Two-stage washing combining sonication, emulsifying agent and chlorine or Tsunami

The average reduction of the total mesophillic count on shredded lettuce was improved (but not significantly, $p=0.05$) for chlorine and tsunami by conducting ultrasonication for 2 minutes with an emulsifying agent (Dipersa-pak, Hydroc™), followed by a sanitised wash with chlorine or Tsunami for 2 minutes (see Figure 8). The total mesophillic counts on shredded lettuce washed in sonicated water containing an emulsifying agent and washed in Tsunami treated water was significantly lower than the unwashed shredded lettuce during storage for 7 days at 4°C.

Figure 8:

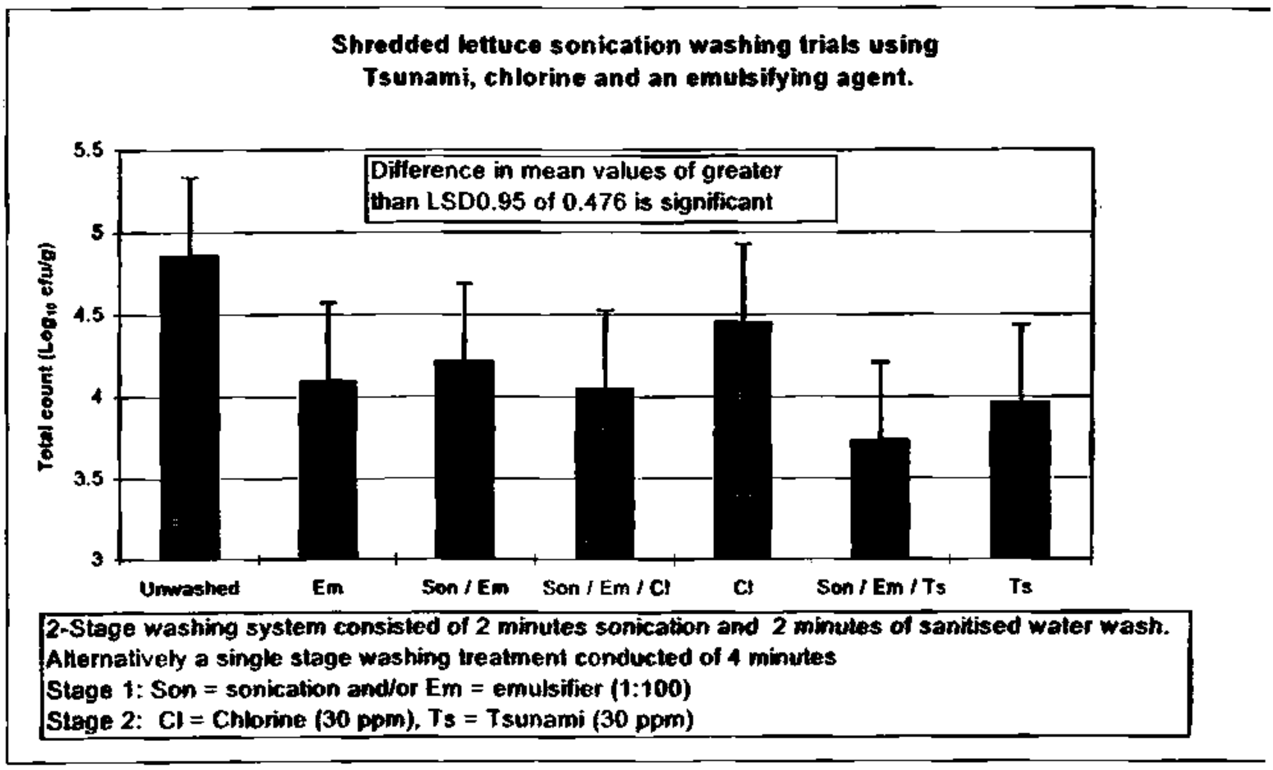
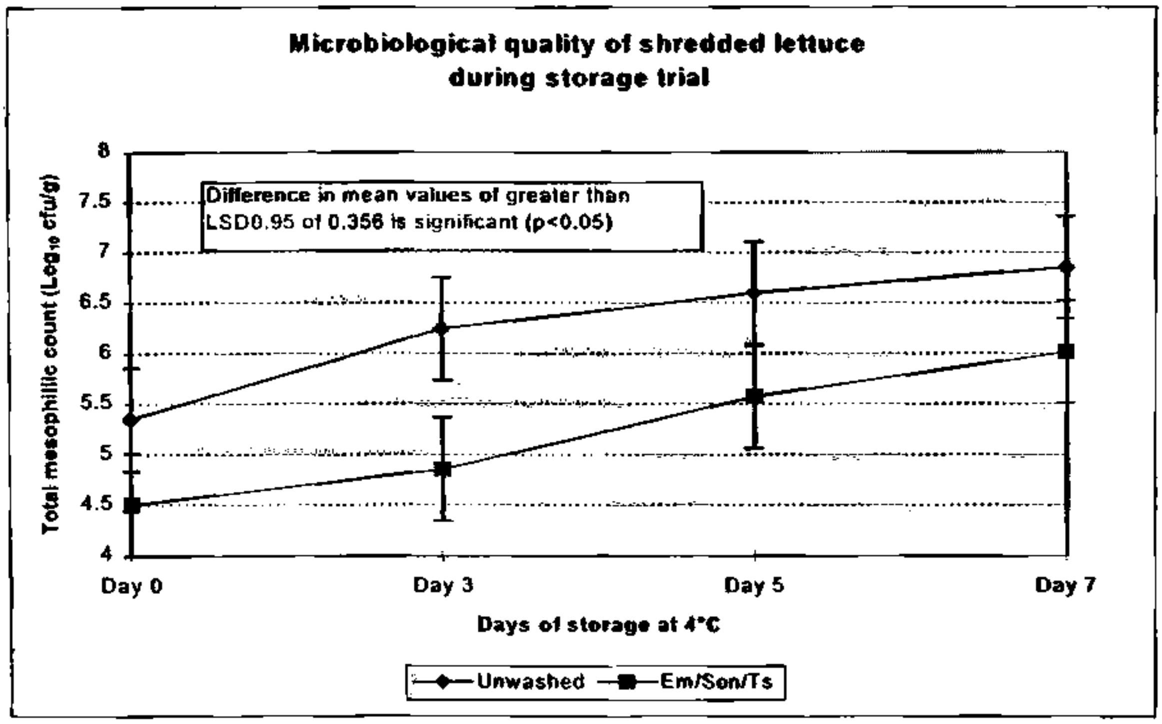


Figure 9:



DISCUSSION

The sanitation of a single batch of shredded lettuce with sonicating water achieved a significant reduction in the total mesophillic count on shredded lettuce. The reduction in the total mesophillic count using chlorine and Tsunami was significantly improved with the sonication of the washing water.

To meet the requirements of fresh-cut processors the sonication system was modified to include water recycling through a heat exchanger to control the temperature of the washing water, while several batches of shredded lettuce were washed.

For the initial batch of shredded lettuce the ultra-sonication was effective in significantly reducing the total microbiological count on the shredded lettuce. However, for subsequent batches of shredded lettuce ultra-sonication was not effective. This may be an effect of the increased organic load in the washing system as monitored by ATP levels. The recycled water could be screened and filtered to reduce the organic load accumulating in the washing system.

RECOMMENDATION

- Conduct further trials using a more advanced ultra-sonication system that can adapt to changes in the wash tank loading.
- Investigation of higher frequency levels to provide more precision in cleaning of intricate surfaces such as lettuce.

Milestone #10: Evaluation of ultrasonic washing for minimally processed vegetables

EXECUTIVE SUMMARY

Ultrasonic washing was evaluated on shredded lettuce, broccoli florets and carrot slices for chilled (4-5°C) and heated (40-50°C) washing applications. Chilled washing was used because it is standard practice for the fresh-cut industry. Heated washing was conducted because it is effective in deactivating browning enzymes.

The total viable reduction when washing in chilled water ranged from 0 to 0.5 Log cfu/g, this was increased to 0.6 to 1.0 Log cfu/g with the application of ultrasonics. Sanitation of the water with peracetic acid at 20 ppm resulted in a total viable reduction ranging from 1.3 to 1.5 Log cfu/g, which was comparable with 1.2 to 1.7 Log cfu/g reduction achieved with the application of ultrasonics. The ultrasonic washing with chilled water (4-5°C) was only marginally effective. Cavitation was not adequately achieved in the chilled washing water, the slight improvement observed in the reduction of the average total viable count for both sanitised and non-sanitised washing was not operationally significant (as established from industry consultation). The total viable count reduction by washing in heated water ranged from 0.3 to 1.4 Log cfu/g, this was increased to 0.7 to 1.8 Log cfu/g with the application of ultrasonics. Sanitation of the water with peracetic acid at 20 ppm resulted in a total viable reduction ranging from 0.5 to 1.8 Log cfu/g, which was increased up to 2.3 Log cfu/g reduction with the application of ultrasonics. The range in the results varied depending on treatment time, ultrasonic operational function and the inherent variation encountered when determining microbiological populations on vegetable produce.

When compared with the industry practice of chilled washing, the application of sanitised ultrasonic washing at 50°C was more effective in reducing the total viable population on shredded, broccoli florets and carrot slices, increasing the Log reduction from 1.1 to 2.2 Log cfu/g, 0.7 to 2.9 Log cfu/g and 1.5 to 2.2 Log cfu/g for each respective product. The reduction in the total viable population was due to the combined effect of heated washing water, sanitation and ultrasonics. Each individual treatment was also demonstrated to provide a greater reduction in the total viable populations.

Deterioration in the quality of the shredded lettuce was observed during storage when ultrasonic washing was performed at 50°C for greater than 1 minute, due to loss of crispness, flaccidity and moisture accumulation. The deterioration in product quality was avoided at 35°C, however this temperature was not effective in reducing the enzymatic browning. At 35°C the application of ultrasonics provided an increased reduction in the total viable number by 0.6 Log cfu/g. An ultrasonic washing application at 40°C for 1 to 1.5 minutes was considered most appropriate to provide the greatest total viable count reduction while still retaining good product quality. The visual quality of the broccoli florets was very good for 3 days of storage, but was unacceptable after 7 days at 4°C, due to excess moisture accumulation, softening and development of an off-odour. An ultrasonic washing application of 40°C for 2 minutes was considered most appropriate to provide the greatest total viable count reduction while still retaining good product quality. The visual and microbiological quality of the carrot slices were both improved with the sanitised-heated ultrasonic washing, due to the reduction of whitening and delay in microbial growth during storage.

With an increasing organic load, a similar rate of decline in peracetic acid concentration was observed with and without ultrasonic application. When the peracetic acid had completely declined due to very high organic loading, the average reduction in total viable population was higher for ultrasonic washing.

Ultrasonic washing of minimally processed vegetables is not suited to chilled washing applications currently conducted by growers and fresh-cut processors. Ultrasonic washing could provide an alternative treatment process, which in combination with mild heat treatment and low-level sanitation to attain a greater reduction in the total viable count on produce, providing appropriate operating conditions are used to prevent deterioration in product quality.

INTRODUCTION

Ultrasonic washing involves generating ultrasonic sound waves by mechanical vibration frequencies greater than 15kHz. The sound waves produce alternating compression and expansion cycles, during which small air bubbles grow until they reach a volume at which they can no longer absorb more energy and implode violently. This phenomenon is known as cavitation.

During 1998-1999 a preliminary investigation into ultrasonic washing of minimally processed vegetables was conducted (submitted to HRDC March 1999, Milestone #5). This investigation demonstrated that the reduction in total viable count on shredded lettuce during sanitised washing (with chlorine and Tsunami) was significantly greater ($p<0.05$) with ultrasonic (25kHz) treatment. The ultrasonic washing was found to be ineffective with increasing organic load, which reduced cavitation. To overcome this limitation an advanced ultrasonic generator was sourced from Branson (US), for trials during 1999-2000 period. This system offered the additional benefits of (i) auto-tuning to compensate for changes in the loading in the washing tank, (ii) line/load regulation to compensate for changes in liquid level and temperature, (iii) variable power control of cavitational intensity allowing for matching to the application, (iv) selectable sweep frequency to reduce standing waves and improve ultrasonic distribution, and (v) power mode used for driving difficult to cavitate systems.

PROJECT AIM

Evaluation and development of ultrasonic washing applications and protocols for minimally processed vegetables.

METHODS

Description of ultrasonication washing system:

A Branson ultrasonic power supply (model 8500) with a frequency output 25 kHz, was used to drive a 15 litre ultrasonic washing tank with 12 in-built transducers. The ultrasonic washing system was leased from Ultrasonics Consolidated (P/L).

Temperature control of washing water

Heating of the washing water was conducted using a coil immersed in a heated washing tank and recirculated at 90 litres per minute. Washing water was chilled by recirculating through a plate heat exchanger connected to chilled water glycol system or pre-chilled in batch tanks within a chiller room.

Product description and preparation:

Washing trials were performed on the following products:

- The outer leaves of iceberg lettuce and the core was removed, then shredded (5mm in width).
- Broccoli florets were cut to size of approximately 5 grams, the main stem was discarded.

- Carrot slices prepared by abrasively peeling carrots and water rinsed to remove peel then sliced (4 mm in width). Carrot batons were processed in the same manner without slicing.

Product was pre-chilled in a cool room at 4°C for about 24 hours prior to processing.

Washing, de-watering and packaging

The product was immersed in the ultrasonicwashing tank for periods from 15 seconds to 5 minutes. Specific immersion times are detailed for each trial in the results section. Product was spin dried for 2 minutes, then 200 grams was packaged into 55 micron polyethylene blend film, with an oxygen transmission rate of 4000 cm^3/cm^2 of film/day.

Monitoring sanitiser levels and washing water chemistry

The peracetic acid component of Tsunami was measured using peracetic acid test strips (Merck P/L))

The pH and oxidative redox potential (ORP) of the washing water was monitored continuously using HP2AS controller (TPS P/L) and 90-Link software system (TPS P/L) for down loading the data. The pH, ORP and temperature compensation probe were inserted into a flume (2 litre volume) which the recirculated water passed trough prior to re-entering the washing tank.

Microbiological analysis

Products were sampled in triplicate for microbiological analysis, 20 gram portions were homogenised (Colworth Stomacher) in 180 mL of 0.1 % bacteriological peptone (Oxoid), then pour-plated or spiral plated with plate count agar (Oxoid) and incubated for 3 days at 30°C.

Water samples were filtered through TTC filter pad (Sartorious), then placed on a moistened nutrient pad and incubated for 1 days at 30°C. Tsunami was neutralised by 1 mL of 1.8% sodium thiosulphate added to the 100mL collection container prior to sampling the washing water.

Statistical Analysis

The mean of 3 replicates, $\pm$ standard deviation, are presented in graphical formats.

RESULTS

Effect of contact time and temperature

Chilled (4-8 °C) ultrasonic washing treatments:

Washing of the shredded lettuce in water alone provided a reduction of the total microbial population in the range of 0 to 0.5 Log cfu/g. A slight increase in the average log reduction of the total microbial population was achieved by ultrasonic treatment of the washing water, which provided a reduction in the range of 0.6 to 1.0 Log cfu/g (see Figure 1). The larger microbial reduction was achieved with using high sweep, high density and amplitude ultrasonic treatments (see Figure 5 & 6). The stated ranges, also included data from ultrasonic contact time trials ranging from 15 seconds to 8 minutes (not presented in graphs).

Chilled (4-8 °C) sanitised ultrasonic washing treatments:

Washing of the shredded lettuce in Tsunami treated water (20 ppm peracetic acid) provided a log reduction of the total microbial population in the range of 1.3 to 1.5 Log cfu/g. Washing of the shredded lettuce in sonicated Tsunami treated water (20 ppm peracetic acid) provided a reduction of the total microbial population in the range of 1.2 to 1.7 Log cfu/g (see Figure 1, 5 & 6).

Heated (45-50 °C) ultrasonic washing treatments:

Washing of the shredded lettuce in heated water provided a reduction of the total microbial population in the range of 0.3 to 1.4 Log cfu/g. The higher microbial reduction was achieved using a treatment time of 2 minutes. Heated (45-50°C) ultrasonic treatment time in excess of 90 seconds resulted in unacceptable damage to the shredded lettuce, resulting in transparent edges 9ie. water logged appearance) and early softening during storage. An increase in the log reduction of the total microbial population was achieved by ultrasonic treatment of the heated washing water, which provided a reduction in the range of 0.7 to 1.8 Log cfu/g. The larger microbial reduction was achieved using a 2 minute treatment time, high sweep, and amplitude ultrasonic treatments (see Figure 5). The microbial reduction with heated sanitised washing water was increased at most by 0.7 Log cfu/g.

Heated (45-50 °C) sanitised ultrasonic washing treatments:

Washing of the shredded lettuce in heated sanitised (Tsunami, peracetic acid 20ppm) water provided a reduction of the total microbial population in the range of 1.3 to 1.8 Log cfu/g. The higher microbial reduction was achieved using a treatment time of 2 minutes. An increase in the average log reduction of the total microbial population was achieved by ultrasonic treatment of the heated washing water, which provided a reduction in the range of 0.5 to 2.3 Log cfu/g. The larger microbial reduction was achieved using a 2 minute ultrasonic treatment. The microbial reduction with heated sanitised washing water was increased on average by 0.6 Log cfu/g and 0.8 Log cfu/g for treatment times of 1.5 and 2 minutes respectively.

Comparison of heated Tsunami washing of shredded lettuce with ultrasonic washing for the same conditions resulted in an increased average reduction in the total viable population for 1.5 minute (1.5 and 2.2 Log respectively), and 2 minute washing periods (1.8 and 2.3 Log respectively) (see Figure 2).

Comparison of heated Tsunami washing of broccoli florets with ultrasonic washing for the same conditions resulted in a comparable average reduction in the total viable population for a 1 minute washing period (1.11 and 1.04 Log cfu/g respectively), a marginal increase at 2 minutes (0.95 and 1.46 Log cfu/g respectively) washing time, and comparable reduction at 5 minutes (1.34 and 1.40 Log cfu/g respectively) see Figure 3).

The heated (50°C) sanitised washing of carrot batons in 20 ppm Tsunami for an extended period (from 1 minute to 5 minutes) resulted in an increase in the average reduction in total viable population (see figure 2). Comparison of heated Tsunami washing of carrot batons with ultrasonic washing for the same conditions resulted in a comparable average reduction in the total viable population for a 1 minute (1.14 and 1.17 Log cfu/g respectively), 2 minute (1.14 and 1.17 Log cfu/g respectively) washing period, and a marginal increase at 5 minutes (1.38 and 1.61 Log cfu/g respectively) (see figure 4).

Operational ultrasonic functions

The average reduction in total viable population was marginally higher for both chilled (7°C) and heated (50°C) washing when using the high sweep frequency-operational function (see Figure 5). In comparison with the other operational functions and taking into consideration the standard deviation of the Log reduction in the total viable population, the operational settings for standard ultrasonic (0.8 & 1.78 Log cfu/g reduction, at 7°C and 50°C respectively), high-sweep (0.94 & 1.88 Log cfu/g) and power mode (0.62 & 1.62 Log cfu/g) were considered comparable. A similar observation was also observed for chilled sanitised ultrasonic washing with a marginally higher average reduction in total viable population (see Figure 6).

Effect of organic load

Increased the organic load in the washing water by washing successive batches of shredded lettuce resulted in the depletion of the peracetic acid concentration (from 30 to 0 ppm) under ultrasonic and non-ultrasonic washing conditions at a similar rate (see Figure 7). During this period the pH of the washing water at commencement was 3.26 and 3.28 for ultrasonic washing and non-ultrasonic washing respectively, which rose to 3.63 and 3.54 respectively after the washing of 6 kg of shredded lettuce. The average microbial reduction declined for Tsunami washing (without ultra-sonication) from 2.25 to 1.5 Log with the depletion of Tsunami. When the Tsunami was depleted during ultrasonic washing, the average microbial reduction remained higher than 2 Log cfu/g (see Figure 7). During the washing period the total count in the water increased approximately 10-fold, with and without ultrasonics (see Figure 8)

Comparison of industry practice and ultrasonic washing system

Ultrasonic washing was conducted in heated sanitised (Tsunami, 30ppm peracetic acid) water at 50°C for 1.5 minutes with shredded lettuce and 5 minutes for broccoli florets and carrot slices. This was followed by a 1 minute rinse in chilled sanitised (Tsunami, 5ppm peracetic acid) water at 4°C. A comparison was made with a 2 step chilled sanitised washing procedure to replicate current industry practice. This involved, chilled sanitised (Tsunami, 30ppm peracetic acid) water at 5°C for 1.5 minutes with shredded lettuce and 5 minutes for broccoli florets and carrot slices. This was followed by a 1 minute rinse in chilled sanitised (Tsunami, 5ppm peracetic acid) water at 4°C. The average microbial reduction was higher with heated sanitised ultrasonic washing compared with the typical industry practice of chilled sanitised washing for shredded lettuce, broccoli florets and carrot slices (see Figure 9).

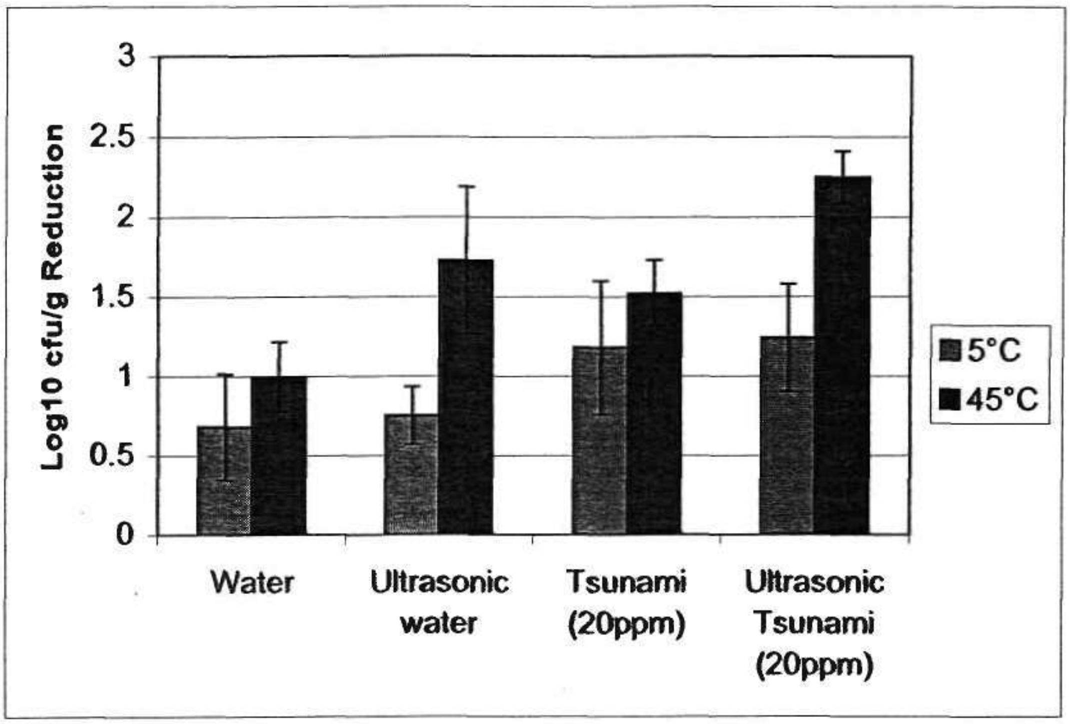
Microbiological shelf-life:

The total viable population on both chilled-sanitised washed and heated-sanitised ultrasonic washed shredded lettuce both grew rapidly from 5.1 and 3.5 to 7.4 and 7.8 Log cfu/g respectively in 10 days after storage at 4°C (see Figure 10). The total viable population on the chilled-sanitised washed broccoli grew rapidly from 4.8 to 6.5 Log cfu/g within 3 days, the maximum population of approximately 7.0 Log cfu/g, which was reached within 7 days. The total viable population on the heated-sanitised ultrasonic washed broccoli florets grew slightly in 3 days from 2.7 to 3.0 Log cfu/g, then grew more rapidly attaining 5.8 Log cfu/g after 10 days (see Figure 11). The total viable population on the chilled-sanitised washed carrot slices remained stable at 2.8 Log cfu/g for 3 days then grew rapidly to 6.5 Log cfu/g after 10 days. The total viable population on the chilled-sanitised washed carrot slices remained stable at 2.0-2.1 Log cfu/g for 3 days then grew slowly from to 2.8 cfu/g after 7 days, then grew rapidly to 6.5 Log cfu/g after 10 days (see Figure 12).

Visual quality shelf-life:

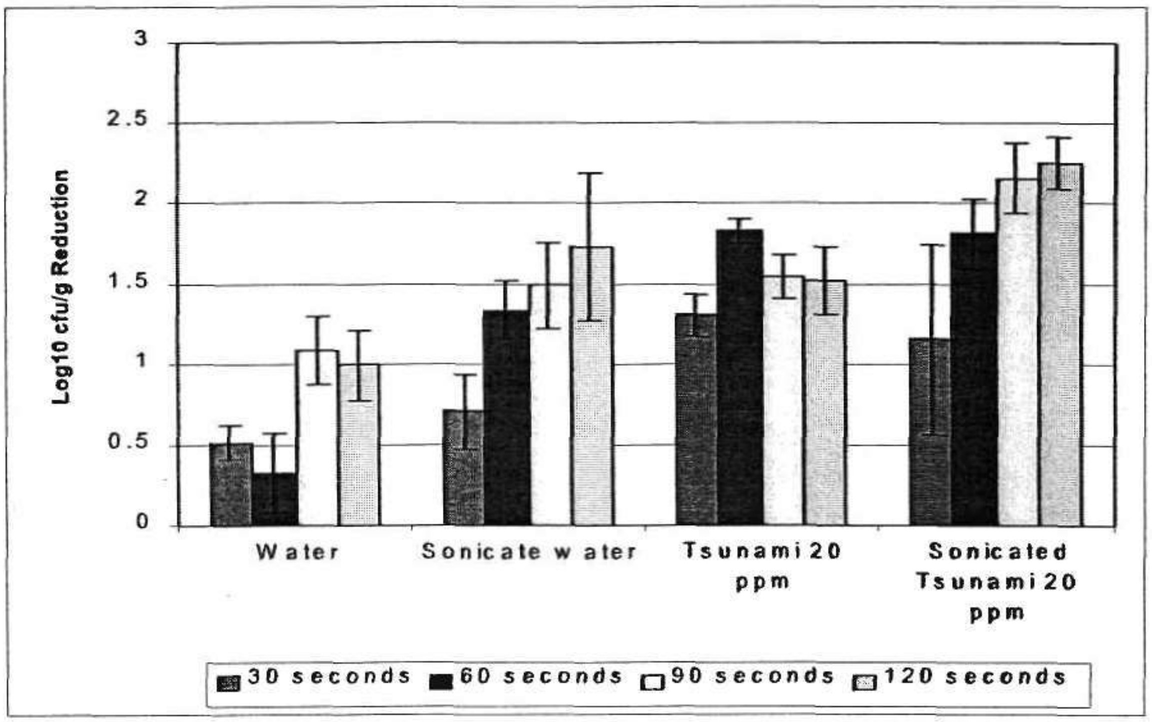
Deterioration in quality of the shredded lettuce was observed during storage for 7 days at 4°C. The ultrasonic washed shredded lettuce became flaccid and softened during storage, and was unacceptable after 7 days, compared with the chilled washed product that remained crisp and in good condition.
The chilled washed broccoli florets became dry and developed moderate whitening on the cut stalk, despite this disorder the product was considered to be of good quality after 10 days of storage. The ultrasonic washed broccoli florets remained moist, as consequence no whitening occurred. The product was of very good quality at 3 days, however by 7 days the moisture increased was unacceptable and by 10 days an off-odour had developed. The chilled washed carrot slices became dry and developed moderate to severe whitening during storage, at 10 days the product was unacceptable. The ultrasonic washed carrot slices remained slightly moist, and no whitening was observed at 10 days the product was in very good quality.

Figure 1. Effect of water temperature on the microbial reduction during non-sanitised and sanitised ultrasonic washing of shredded lettuce



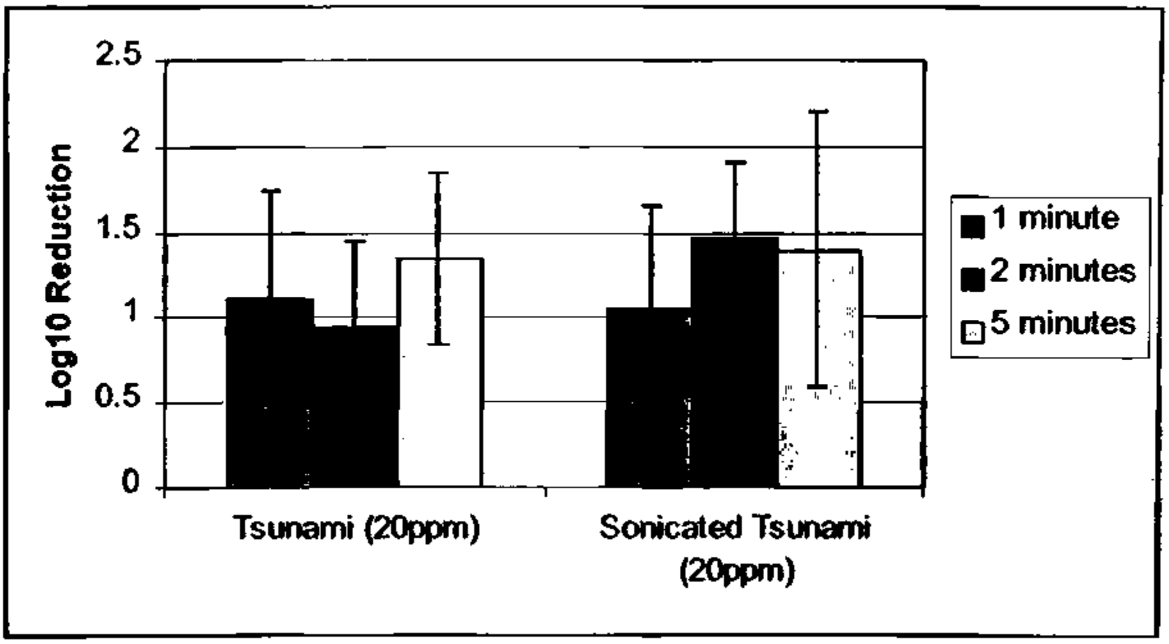
Note: Tsunami concentration at 20 ppm peracetic acid

Figure 2. Effect of contact time on microbial reduction during heated non-sanitised and sanitised ultrasonic washing of shredded lettuce



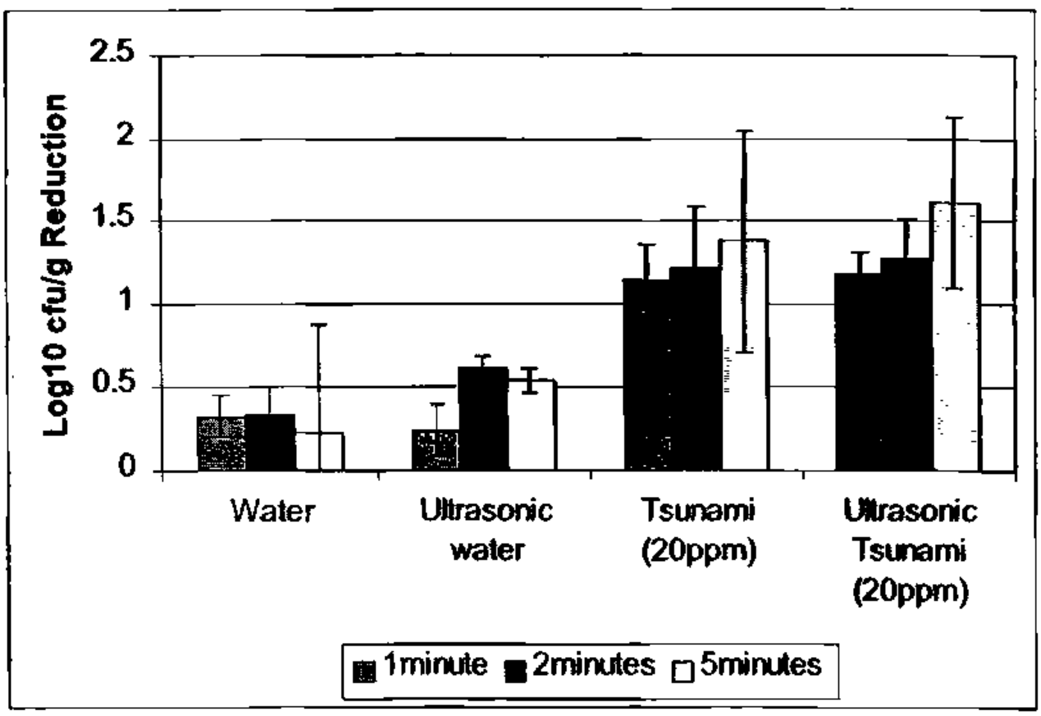
Note: Tsunami concentration at 20 ppm peracetic acid

Figure 3. Effect of contact time on the microbial reduction during heated (50°C) ultrasonic washing of broccoli florets



Note: Tsunami concentration at 20 ppm peracetic acid

Figure 4. Effect of contact time on the microbial reduction during chilled (50°C) ultrasonic washing of carrot batons



Note: Tsunami concentration at 20 ppm peracetic acid

Figure 5. Effect of ultrasonic operating functions on the microbial reduction during the washing of shredded lettuce

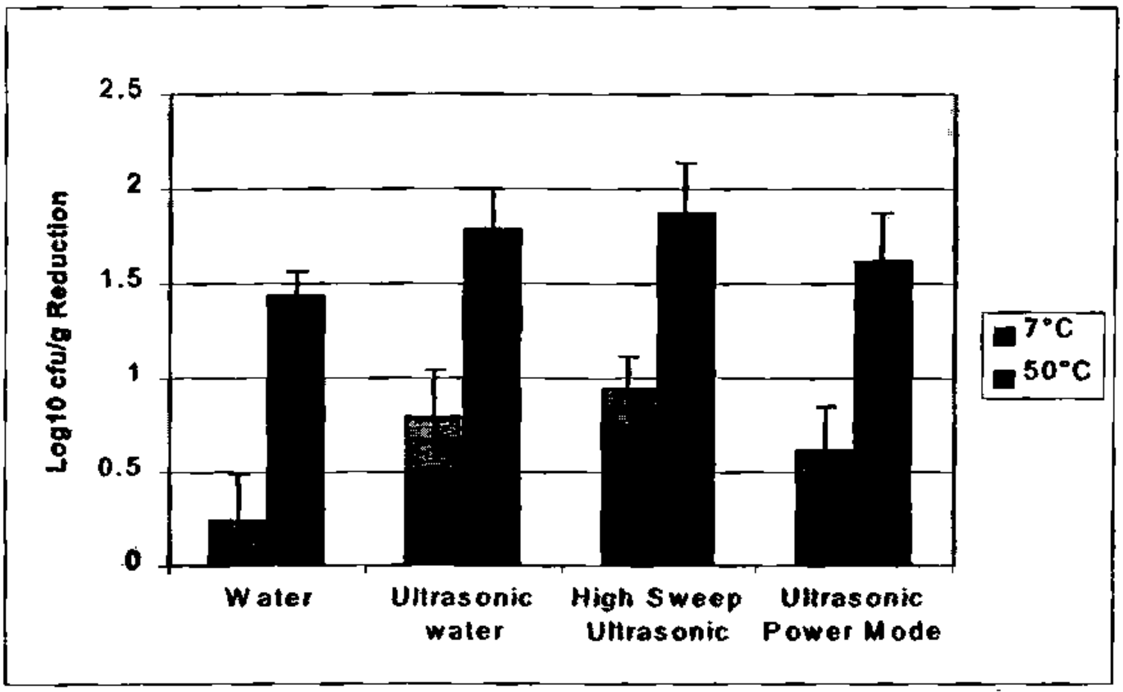
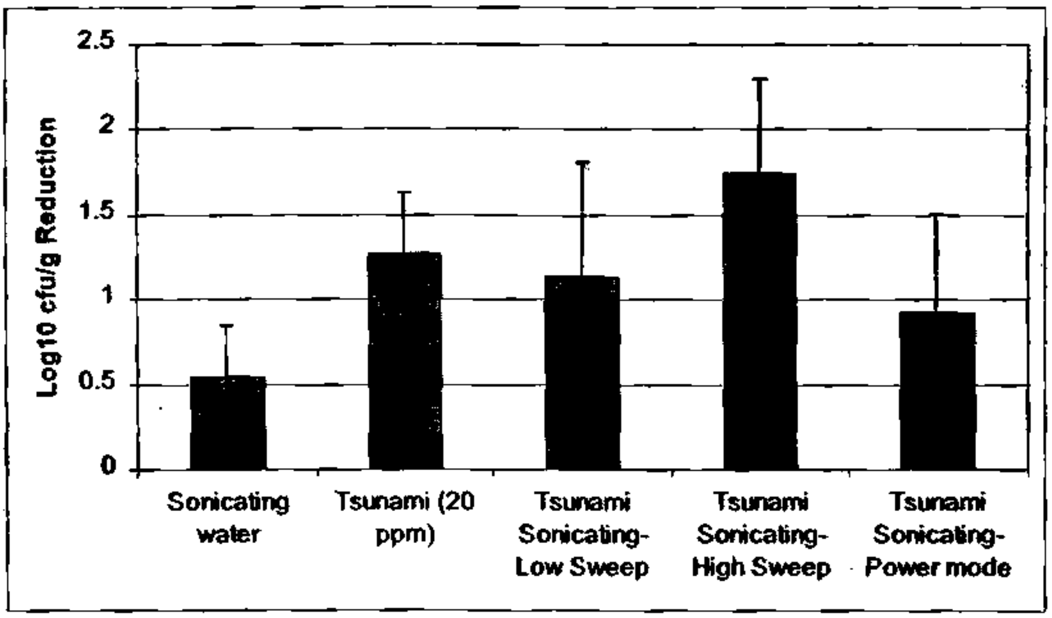


Figure 6. Effect of ultrasonic operating functions on the microbial reduction during chilled (4-5°C) sanitised washing of shredded lettuce



Note: Tsunami concentration at 20 ppm peracetic acid

Figure 7. Effect of increased organic load on the microbial reduction during heated (50°C) sanitised ultrasonic washing of shredded lettuce

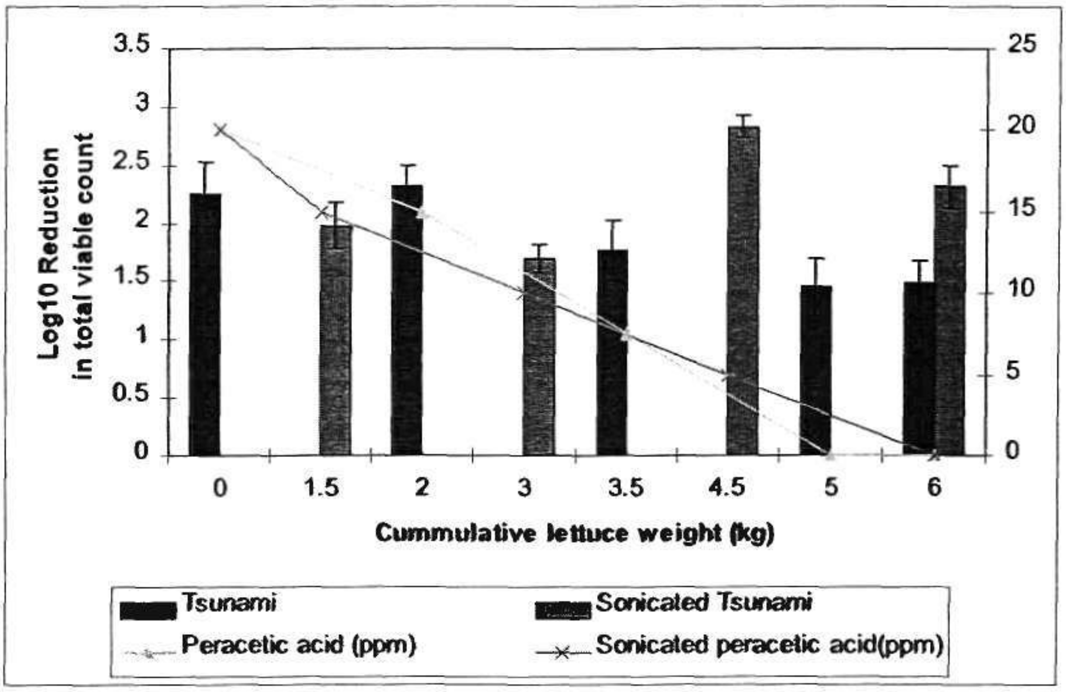


Figure 8. Effect of increased organic load on the microbial count in the washing water during heated (50°C) sanitised ultrasonic washing of shredded lettuce

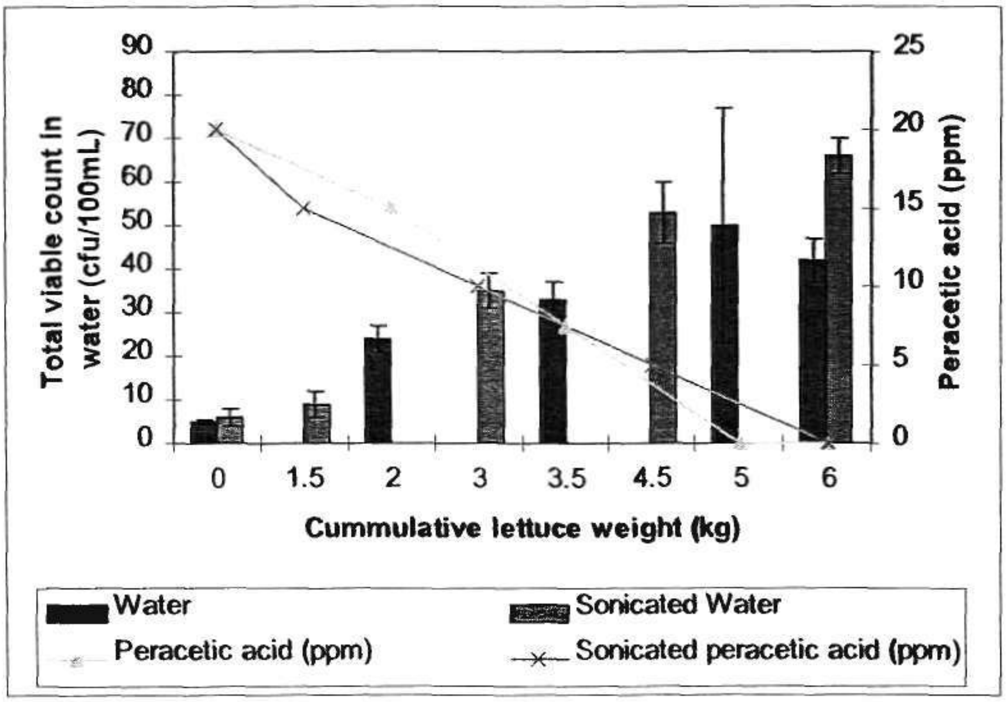
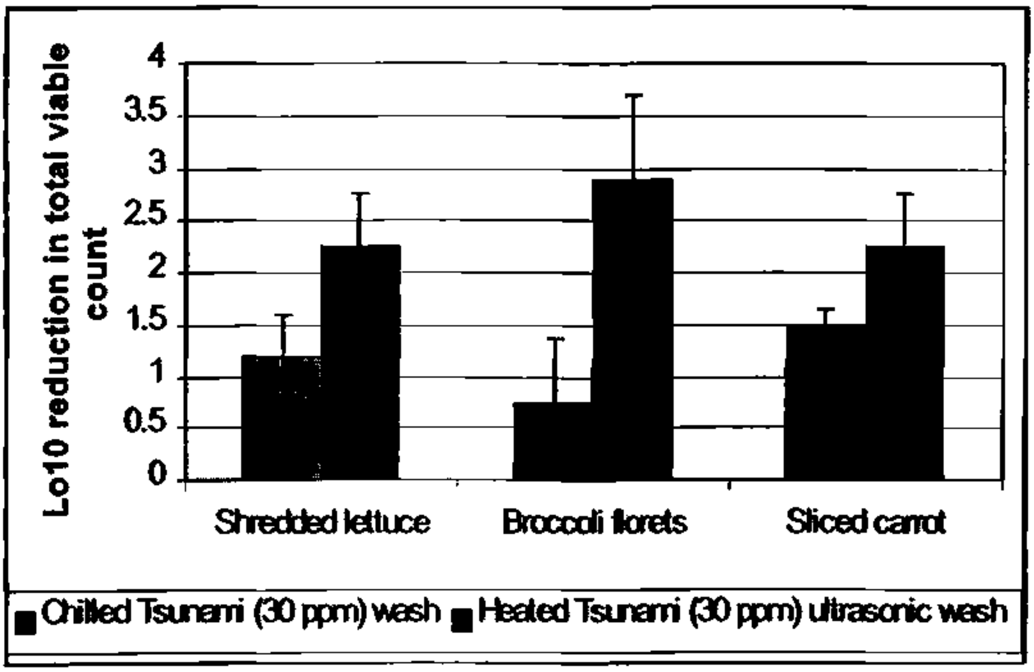


Figure 9. Comparison in the microbial reduction between industry practice of chilled (4°C) sanitised washing and sanitised heated (50°C) ultrasonic washing for shredded lettuce, broccoli florets and carrot slices.



Note: Tsunami concentration at 20 ppm peracetic acid. Two stage washing process; shredded lettuce washed for 90 seconds, broccoli and carrots washed for 5 minutes then followed by rinse for 1 minute in chilled water (4°C).

Figure 10. Total viable counts on shredded lettuce during storage at 4°C.

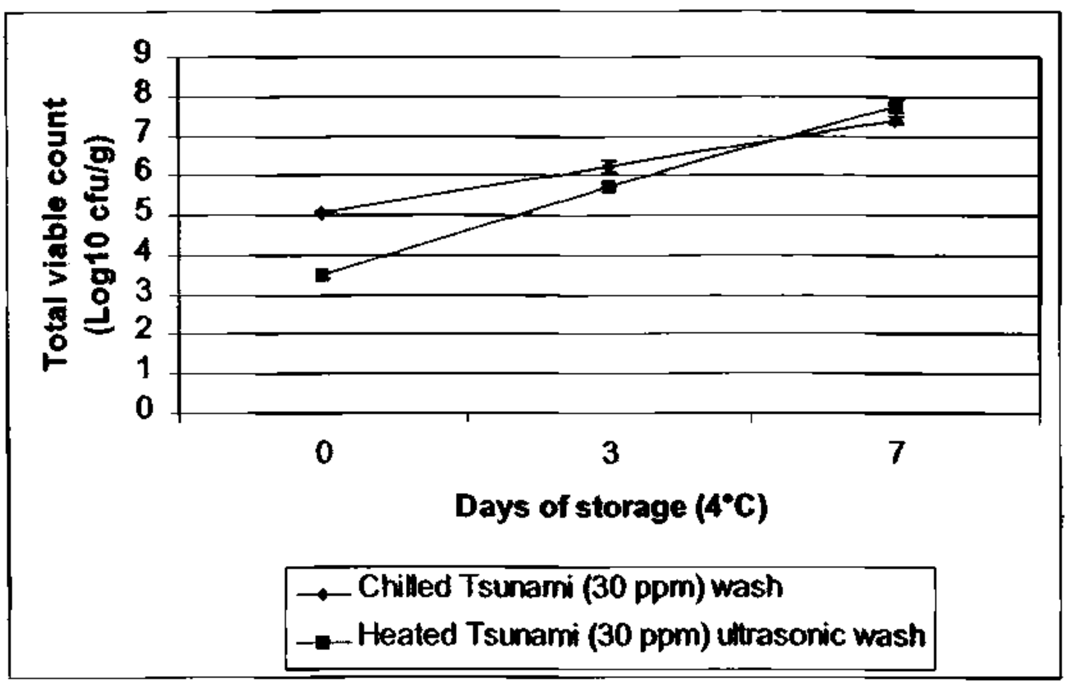


Figure 11. Total viable counts on broccoli florets during storage at 4°C.

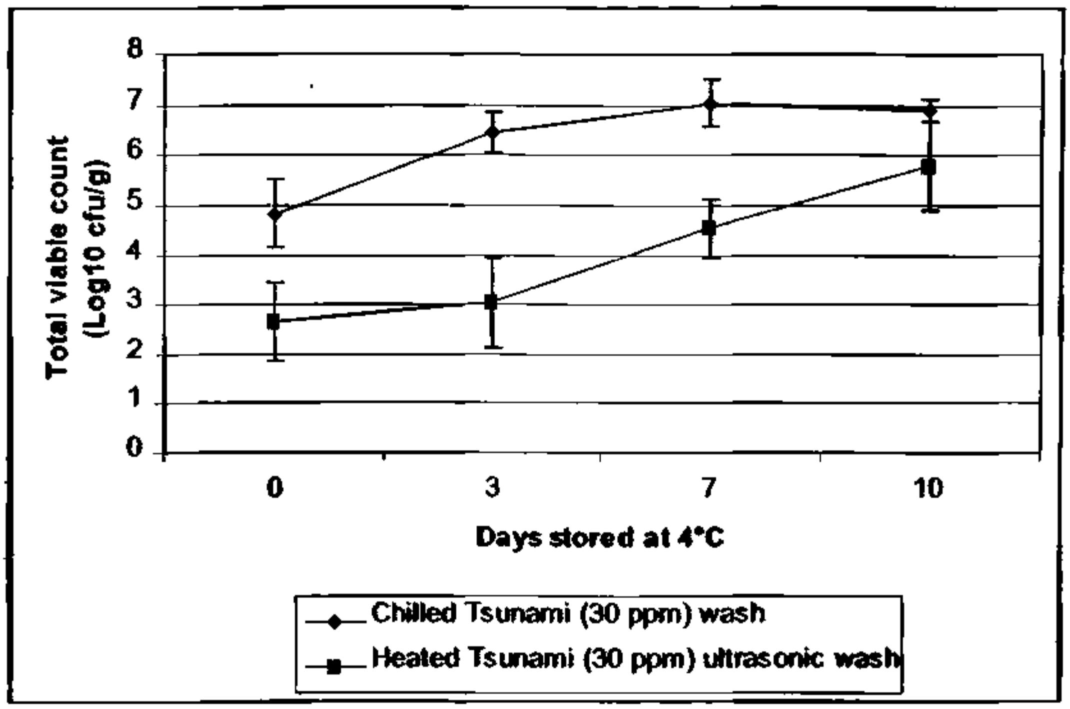
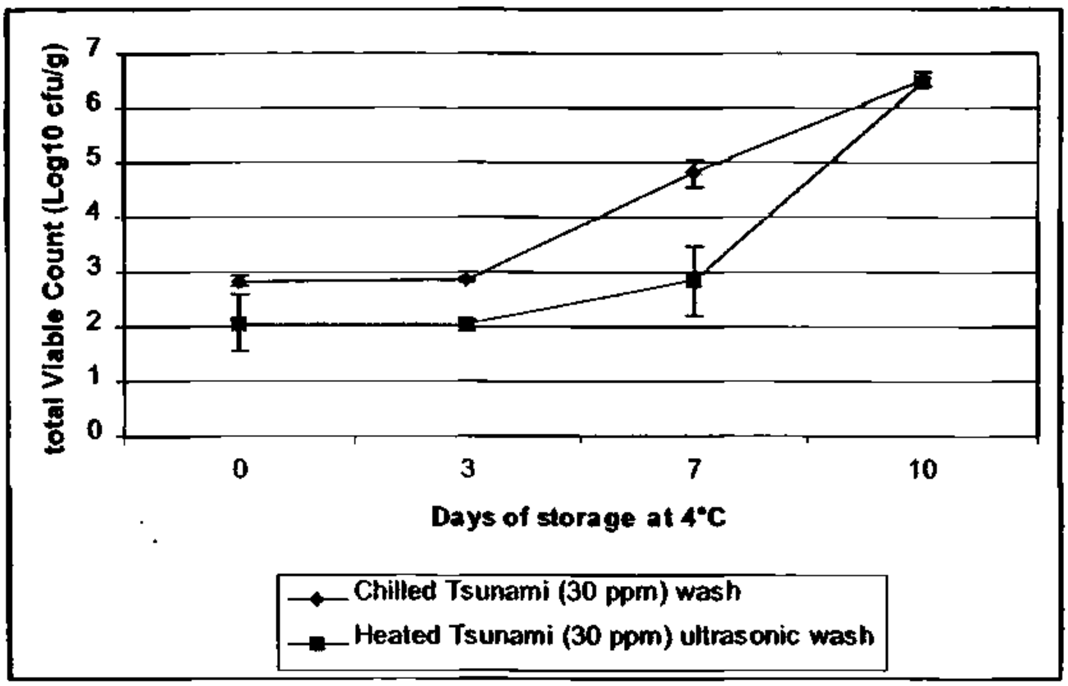


Figure 12. Total viable counts on carrot slices during storage at 4°C.



DISCUSSION

The washing of minimally processed vegetables generally involves the use of chilled sanitised water (2°C to 8°C). Chilling of the washing water is performed to retain product quality by reducing the potential for microbiological growth, enzyme activity and product respiration during processing.

The ultrasonic washing was not effective when used in chilled water (4-5°C). Cavitation was probably not adequately achieved in the chilled washing water, the slight improvement observed in the reduction of the average total viable count for both sanitised and non-sanitised washing was not operationally significant (as established from industry consultation).

Washing of the lettuce at 45-50°C has been reported to reduce the browning on shredded lettuce (Loaiza-Verlarde, J.G., 1997 and Delaquis, P.J., et al., 1999). As this approach provided an alternative treatment for the washing of minimally processed vegetables and a more suitable temperature to apply ultrasonic washing, trials were conducted at 45-50°C. When compared with the industry practice of chilled washing, application of sanitised ultrasonic washing at 50°C was more effective in reducing the total viable population on shredded lettuce, broccoli florets and carrot slices (see Figure 9). The carrot batons were pre-washed during abrasive peeling prior to the sanitised ultrasonic washing trial, consequently the microbial reduction was generally low. The reduction in the total viable population was due to the combined effect of heated washing water, sanitation and ultrasonics. Each individual treatment also was demonstrated to provide a greater reduction in the total viable populations (see Figure 1). These findings are comparable to the additional 1 Log cfu/g reduction reported with ultrasonic washing when using 100 ppm chlorinated water from Campden & Chorleywood Food Research Association (personal communication, Dr Ian Seymour). These findings were conducted on a smaller 2 litre system (15-20 litres used in this project), which is presently being scaled up to a 40 litre system.

The quality of the lettuce washed in sanitised heated ultrasonic water was inferior to the sanitised chilled washed lettuce. This was partially an effect of ultrasonic washing time and washing temperature. A shorter ultrasonic washing period of 1 minute or less reduced the product damage however did not provide improved reduction in the total viable count (see Figure 2). The deterioration in product quality from the effect of heated ultrasonics was avoided at 35°C, however this temperature was not effective in reducing the enzymatic browning. At 35°C the application of ultrasonics provided an increased reduction in the total viable number by 0.64 Log cfu/g. An ultrasonic washing application of 40°C for 1 to 1.5 minutes is likely to provide the greatest total viable count reduction while still retaining good product quality.

The visual quality of the broccoli florets was very good for 3 days of storage, but rapidly deteriorated to be unacceptable after 7 days at 4°C. In consideration of the results with shredded lettuce, the application of ultrasonics on broccoli at a lower temperature (40°C) for a shorter treatment time should be beneficial for product quality, but this was not evaluated.

The visual and microbiological quality of the carrot slices were both improved with the sanitised-heated ultrasonic washing. The application of high temperature and acid treatment has been previously demonstrated to inhibit the development of whitening on carrot surfaces (Bolin, H.R. and Huxsoll, C.C., 1991). Ultrasonic washing may be applicable for stronger textured vegetable products such as potatoes and pumpkins.

With an increasing organic load, a similar rate of decline in peracetic acid concentration was observed with and without ultrasonic application (see Figure 7). When the peracetic acid had been completely declined, the average reduction in total viable population was higher for ultrasonic washing (see Figure 7). The peracetic acid breaks down to acetic acid carbon dioxide and water. The presence of the acetic acid maintained a reduction in the total viable population that was greater then that normally achieved with water. In addition, the acidified water would have also assisted in limiting the total count in the washing water to below 100 colony forming units per 100mL.

CONCLUSIONS

Ultrasonic washing of minimally processed vegetables is not suited to the general chilled washing applications currently conducted by growers and fresh-cut processors. Ultrasonics could be applicable to situations were the product is undertaking a mild heat treatment to facilitate cavitation and where the application of sanitisers was to be avoided. The treatment of carrot slices was identified as a suitable product suitable for heated ultrasonic washing with peracetic acid.

References

Bolin, H.R. and Huxsoll, C.C. (1991). Control of minimally processed carrot surface discolouration caused by abrasive peeling. J. Food Sci. 56: 416-418.

Delaquis, P.J., Stewart, S., Toivonen, A.L. and Moyls, A.L. (1999) Effect of warm chlorinated water on the microbial flora of shredded iceberg lettuce. Food Research International 32: 7-14.

Loaiza-Verlarde, J.G. (1997) Effect of intensity and duration of heat-shock treatments on wound-induced phenolic metabolism in iceberg lettuce. Journal of American Society for Horticulture Science 122(6): 873-877.