Ultrasound decontamination of minimally processed fruits and vegetables

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Summary The effectiveness of power ultrasound for the microbial decontamination of minimally processed fruits and vegetables was studied. Reductions in *Salmonella typhimurium* attached to iceberg lettuce obtained by cleaning with water, chlorinated water, ultrasound with water and ultrasound with chlorinated water were 0.7, 1.7, 1.5 and 2.7 logs, respectively, for small-scale (2 L) trials. The cleaning action of cavitation appears to remove cells attached to the surface of fresh produce, rendering the pathogens more susceptible to the sanitizer. For large-scale (40 L) trials, the addition of chlorine to water in the tank gave a systematic difference in *Escherichia coli* decontamination efficiency. However, the frequency of ultrasound treatment (25, 32–40, 62–70 kHz) had no significant effect on decontamination efficiency ($P > 0.69$). With the potentially high capital expenditure together with the expensive process of optimization and water treatment, it is unlikely that the fresh produce industry would be willing to take up this technology. Furthermore, the additional one log reduction achieved by applying ultrasound to a chlorinated water wash does not completely eliminate the risk of pathogens on fresh produce.

Keywords Decontamination, fruits and vegetables, food borne pathogens, food safety, minimally processed, ultrasound.

Introduction

Minimally processed refrigerated (MPR) fruits and vegetables are fresh, raw foods which are usually processed and sold to consumers in a ready-to-eat form (Wiley, 1994). The presence of spoilage bacteria, yeasts and moulds and the occasional pathogen on fresh produce has been recognized for many years (Beuchat, 1998). MPR products support the survival and/or growth of food borne pathogens as the fresh nature of the produce combined with mild processing and storage regimes can present potential infection vehicles (Francis *et al*., 1999), and have been implicated in a number of food poisoning incidents (Nguyen-the & Carlin, 1994; Beuchat, 1998).

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At present, cleaning technologies cannot guarantee the microbiological safety of MPR products without compromising their quality (Seymour, 1999). Effective washing and decontamination of fruits and vegetables is difficult because attached or entrapped bacteria are not readily accessible to biocides. Washing generally involves the immersion of product in cooled sanitized water (Simons & Sanguansri, 1997). Vigorous washing in potable water typically reduces the number of microorganisms by 1–2 logs and is often as effective as treatment with 100 ppm chlorinated water, the current industry standard (Beuchat, 1998; Seymour, 1999). Commercial washing systems are highly variable, removal of dirt and foreign matter is variable, pesticide removal is limited and shelf life extension by washing is almost impossible (Seymour, 1999). Therefore, current methods for fresh produce decontamination may be relatively

ineffective for pathogen removal and more effective treatments are required. Power ultrasound, as used for cleaning in the electronics industry has a potential application to fresh produce decontamination. Power ultrasound (US) is used at frequencies in the range 20–100 kHz and requires the presence of a liquid medium for power transmission (reviewed by Roberts, 1991; Mason, 1993). Ultrasonic fields consist of waves at high amplitude, which form cavitation bubbles (Scherba *et al*., 1991). The growth, collapse and oscillation of these bubbles generate the mechanical energy which has a 'cleaning' action on surfaces (Kinsloe *et al*., 1954; Roberts, 1991; Scherba *et al*., 1991; Schett-Abraham *et al*., 1992; Earnshaw *et al*., 1995; Sala *et al*., 1995; Raso *et al*., 1998). Several workers have examined the antimicrobial effects of ultrasound on bacteria (Ordonez *et al*., 1987; Scherba *et al*., 1991; Raso *et al*., 1998; Pagan *et al*., 1999), spore formers (Sanz *et al*., 1985) and moulds (Idrissi *et al*., 1996). Nevertheless, comparisons are difficult to make because of significant variations in physical parameters, such as ultrasound frequency, power level, the size and shape of the ultrasonic bath, the depth, volume temperature and nature of the liquid, and treatment time (Jeng *et al*., 1990). Ultrasound has recently been proposed for use in food preservation but this has not been adopted because of the perceived adverse effects on food quality brought about by the high-intensity treatments required to inactivate the most resistant micro-organisms.

The objective of the present study was to investigate the potential of ultrasound for the inactivation and/or removal of bacteria attached to the surfaces of fruits and vegetables.

Materials and methods

Bacterial strains and growth conditions

The strains used were *S. typhimurium* NCIMB 10248, *Listeria monocytogenes* [Campden Research Association (CRA) 1177] and *E. coli* NCIMB 12497 (Ampicillin resistant). Cultures were maintained on glass beads at −80 °C in glycerol/water (1:1 v/v). Bacteria were resuscitated by placing one bead in 10 mL of Nutrient Broth (NB) followed by incubation at 37 °C for 24 h. These primary cultures were streaked on Nutrient Agar (NA), incubated at 37 °C for 24 h, and stored at 4 °C prior to use. Experimental cultures were prepared by inoculating NB (90 mL) with a single colony from an NA plate followed by 24 h incubation at 37 °C. For *E. coli* NCIMB 12497, all growth media were supplemented with 50 μg mL^{-1} of ampicillin (Sigma, St. Louis, USA).

Enumeration of bacteria

After treatment, fresh produce samples (10 g) were diluted with 90 mL of Maximum Recovery Diluent (MRD; 0.1% peptone + 0.85% NaCl) and then homogenized in a stomacher (Laboratory-blender 400, Seward, UK) for 120 s. Serial dilutions were prepared in MRD and surviving micro-organisms were enumerated. *Salmonella* spp. were enumerated by spread plating on Xylose Lysine Deoxycholate Agar (XLD; Oxoid, CM469) and incubated at 37 °C for 24 h. *Escherichia coli* was enumerated by pour plating on Violet Red Bile Lactose Agar plus 50 μg mL^{-1} ampicillin (VRBA; Oxoid, CM485) with overlay and then incubated at 37 °C for 24 h.

Preparation of inoculated fruits and vegetables

Fresh produce items (iceberg lettuce, whole cucumber, cut baton carrot, capsicum pepper, white cabbage, spring onion, strawberry, curly leaf parsley, mint and other herbs) were purchased from a local supermarket. Reference materials were either left whole (uncut) or trimmed and sliced (cut) by hand using aseptic techniques. The only products not to be tested cut were carrot and cucumber. All products were stored below 4 °C prior to treatment.

Fresh produce items were inoculated with known levels of bacteria as described by Zhang & Farber (1996). Approximately 100 g of prepared fruits and vegetables were placed into a sterile Stomacher^{R}. Laboratory System plastic bag (Model 400, Seward; 19 × 30 cm). Each bag was inoculated with 1 mL of bacterial culture (approximately 10^{8} cfu mL^{-1}), heat-sealed, and then shaken gently thirty times to ensure an even distribution of the organism on the product. To allow sufficient time for microbial attachment, the samples were stored overnight at 4 °C. This gentle shaking did not damage the products. Inoculated

fresh produce samples were removed with the aid of sterile forceps and then drained for 1 min. Remaining micro-organisms were enumerated before and after treatment to assess bacterial attachment.

Assessment of bacterial attachment

Cut and uncut fruits and vegetables (100 g) were inoculated with 10^6 cells g^{-1} of *S. typhimurium* and washed for 5 min in 1 L sterile tap water in a 5-L beaker with constant agitation (product to water ratio 1:10). After treatment, samples were removed with the aid of sterile forceps and then drained for 1 min. Remaining bacteria were enumerated before and after washing to assess bacterial attachment ($n = 5$).

Chemicals

Sodium hypochlorite (Sigma) containing 4–20% available chlorine was made up to the required concentration in sterile distilled water (SDW). The pH was adjusted to 7.0 ± 0.1 with HC1 or NaOH. Free residual chlorine was measured using the Palintest® chlorine test kit. Ten parts per million of the food grade surfactant, dioctyl sodium sulphosuccinate (Sigma) was also used in combination with sodium hypochlorite for ultrasound trials.

Chlorine test

The Palintest® Comparator (Model PT 520T, Palintest Ltd, Gateshead, UK) was used to test the active concentration of chlorine. Using different reagents, this test detects both total and free chlorine [Chlorine DPD (Comp. 7, Palintest Ltd, UK), 0–5.0 mg L^{-1} free and total chlorine; Chlorine HR (Comp.8, Palintest Ltd, UK), 0–250 mg L^{-1} total chlorine].

Assessment of product breakdown

Tests were made by using a range of ultrasound tanks (with nominal capacities between 35 and 45 L) supplied by Kerry Ultrasonics Ltd. Various treatment times were used at frequencies of 25, 32–40 and 62–70 kHz and a power up to 15 W L^{-1}. The ultrasound cleaning systems were set up as described in the Operation and Maintenance manual (Kerry Ultrasonics Ltd, Hunting Gate, Wilbury Way, Hitchin, Herts., SG4 0TQ, UK). Ten samples from each batch of food were used and items of food were treated individually.

High power bar and radial applicators, both operating at 20 kHz, were also used (FFR Ultrasonics Ltd, Leics, UK). The bar applicator was operated at 450 W in to 2 L of water containing a small sample of food located beneath the bar. The nominal power was 225 W L^{-1}. However, the ultrasound field was probably quite localized (as seen from the rippling of the surface of the water near the bar). Based on the 'footprint' of the bar which measured 140 by 38 mm and the height above the base of the container (25 mm), the volume of liquid beneath the bar was 133 mL. Assuming that the ultrasound field is restricted to the liquid directly below the bar, the power input to the liquid and food material would be 3400 W L^{-1}. The radial cell operated at powers up to 1200 W. In general, the product flows through the centre of the cell but in these tests the product and water were sealed into the cell using tape. The cell contained approximately 40 mL of water and a small sample of the food. The power density was approximately 30 000 W L^{-1}.

Small scale ultrasound decontamination trials

Aseptic conditions were maintained throughout all the experiments to ensure accurate assessment of fresh produce decontamination. An ultrasound tank (KS450, Kerry Ultrasonics Ltd) was filled with 40 L of sterile tap water (STW) and degassed at an operating frequency of 32–40 kHz (10 W L^{-1}) for 10 min. A 5-L capacity stainless steel sub-bath was placed in the large tank and filled with 2 L of STW with/without 25 ppm free residual chlorine (pH 7.0 ± 0.1). Cut iceberg lettuce (100 g) inoculated with 10^6 cells g^{-1} of *S. typhimurium* was placed in a stainless steel wire basket and submerged in the sub-bath containing the various test solutions (product to water ratio 1:20). The product was washed for 10 min with/without ultrasound treatment at 32–40 kHz (10–15 W L^{-1}). Remaining *S. typhimurium* cells were enumerated before (in triplicate) and after washing (in quintuplicate) to assess the decontamination efficiency.

The four treatments are summarized as follows: (1) tap water dip (control, no agitation) (2) 25 ppm free chlorine dip, (3) ultrasound only, and (4) ultrasound and 25 ppm free chlorine.

Large scale ultrasound decontamination trials

Disinfected ultrasound tanks (KS450, Kerry Ultrasonics Ltd) were filled with 40 L of tap water and degassed at the operating frequency to be used in the subsequent trials (25, 32–40, 62–70 kHz, 10–15 W L^{-1}) for 10 min. Stannard (1997) described acceptable microbiological limits for raw and prepared vegetables (including salad vegetables). Although high levels of Aerobic Plate Counts (APC), Enterobacteriaceae and coliforms are probable, they may be derived from the soil or poor handling, and do not give a true measure of the microbiological safety. Alternatively *E. coli* is thought to be a more appropriate indicator organism for the presence of faecal contamination (Stannard, 1997). The strain *E. coli* NCIMB 12497 with ampicillin resistance as a selectable marker was used for all large-scale ultrasound decontamination trials. By using this strain in combination with the appropriate selective media, the levels of *E. coli* remaining on the fresh produce were routinely monitored (removing high background counts of Enterobacteriaceae and coliforms). Samples (200 g) of either cut iceberg lettuce, curly leaf parsley, strawberry or cabbage were inoculated with 10^6 cells g^{-1} of *E. coli* NCIMB 12497, placed in a stainless steel wire basket and submerged in the ultrasound tank containing 40 L of the various test solutions. A product to water ratio of 1:200 is not typically used in industry but it was important to present the foodstuffs in the treatment tank as a single layer to optimize cavitation action. The total and free chlorine levels were monitored before and after washing. The products were washed for 10 min under five treatment regimes. These are summarized as follows: (1) tap water dip (control, no agitation) (2) 100 ppm free chlorine dip (pH 7.0 ± 0.1) (3) ultrasound only (4) ultrasound and 100 ppm free chlorine (5) ultrasound, 100 ppm free chlorine and 10 ppm dioctyl sodium sulphosuccinate. Remaining *E. coli* cells were enumerated before (in triplicate) and after washing (ten replicates) to assess the decontamination efficiency. For all four products, ultrasound treatments (3) to (5) were used at three operating frequencies (25, 32–40, 62–70 kHz; 10 W L^{-1}).

Results

Assessment of bacterial attachment

The attachment efficiency of *S. typhimurium* on a range of cut and uncut fresh produce items is shown in Fig. 1. There was a difference in the attachment efficiency of *S. typhimurium* on different fresh produce types. For example, reductions of 1.0, 1.3 and 1.5 logs of *S. typhimurium* were

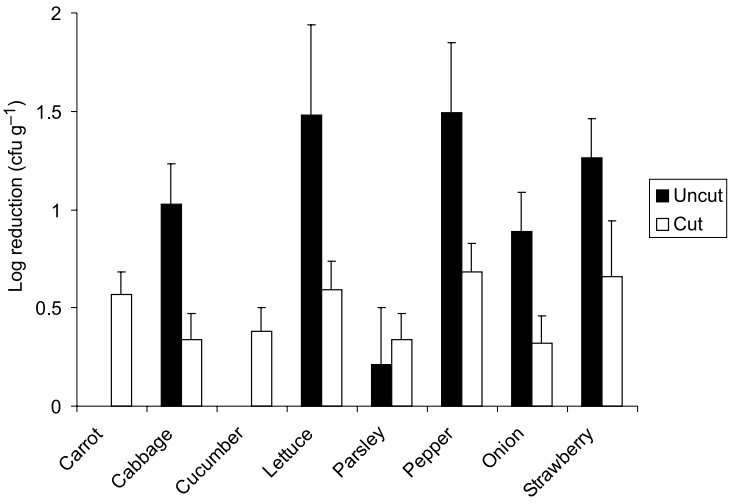

Figure 1 The effects of washing in potable water on the decontamination efficiency of *Salmonella typhimurium* on cut and uncut fresh produce. Remaining bacteria were enumerated before and after washing to assess bacterial assessment ($n = 5$). Fresh produce samples uncut (–) or cut (?). Uncut carrot and cucumber were not tested.

observed on uncut cabbage, strawberry and pepper, respectively. However, uncut spring onion and parsley demonstrated less than a 1.0 log reduction when washed for the same length of time under identical conditions. These results suggest that sufficient numbers of S. *typhimurium* cells were becoming attached to the products during the inoculation protocols. In general, if the surfaces were cut the log reductions achieved were lower than for the uncut surfaces therefore suggesting that bacterial attachment is greater for the cut products compared with the corresponding uncut products. Experiments were also repeated for *E. coli* and *Listeria monocytogenes* (iceberg lettuce) but no significant differences in attachment efficiency were evident (results not shown).

Tests to assess the breakdown of foods

The breakdown of fruit and vegetable products was assessed under different frequency/power/time combinations in ultrasound tanks. Irrespective of frequency, only the mint showed any notable signs of breakdown after 300 s, characterized by a loss of colour very close to the edge of each leaf. All other tested products were visibly fresh and undamaged, even after 600 s treatment. Further tests used a 35-kHz ultrasound system (Kerry Ultrasonics Ltd, UK), but operating beyond its design range to achieve a power of 50 W L^{-1}. This could be maintained for only 120 s without the risk of damage to the equipment. Again, mint was the only product to show any signs of breakdown. These tests found no effect of time, frequency or power input on damage to the foods, other than mint, over the range of the tests. Further tests were made by using materials stored for 4 days after purchase at 4 °C and then treated for 300 s. The mint exhibited signs of breakdown as in previous tests while very slight damage at the edges of lettuce leaves and parsley was also noted.

Preliminary investigations were made by using high power bar and radial applicators. The 'hairs' on strawberries were removed after a 30-s bar applicator treatment but no other visible changes were apparent. Similar results with mint and strawberries were found after 150 s, lettuce appeared slightly blotchy while parsley demonstrated slight darkening at the edges. After treatment for 300 s, the carrot, pepper and spring onion were the only products to show no visible signs of damage. After a 1-s radial applicator treatment, the extreme edge of cabbage leaves exhibited a slight change in colour, the lettuce leaves displayed patchy areas while the pepper and spring onion demonstrated no visible effects of treatment. Some hairs were dislodged from the surface of the strawberries. Increasing the treatment time merely led to more visible changes. After 10 s, the carrot, pepper and spring onion showed no visible change but the water was slightly coloured indicating that some surface damage had occurred.

The optimum water temperature for ultrasound cleaning is around 60 °C. Operating at this temperature would not be feasible with fresh fruit and vegetables as it would affect the food texture and enhance deterioration of sensory qualities. Water at a starting temperature of either 5 or 20 °C had no different effects on the breakdown of foods after 300 s treatment in the ultrasound tanks. Significant heating during an ultrasound treatment could have deleterious effects on food quality. With the ultrasound tank, the water temperature increased by approximately 0.2–0.3 °C min^{-1}. This compared with a 3.2 °C min^{-1} and a 2.6 °C s^{-1} (equivalent to 156 °C min^{-1}) increase for the bar and radial applicators, respectively. As expected, the rate of change of water temperature was very much dependent on the power applied. The difference between the measured power and nominal (stated) power of the tank systems is generally small. However, the more intense bar and radial applicators became hot and it is unlikely that all of the energy was transferred to the water. All tests were done with the water and ultrasound equipment initially at 20 °C.

The chemical composition of the water in the treatment tank can have an effect on the action of ultrasound. Tests using a tank system (operating up to 25 W L^{-1}) used tap water from three sources (Clarkes water hardness values of 20, 23.4 and 24.19, respectively). De-aerated water, alcohol in tap water (2% v/v), and tap water with a surfactant (0.05% v/v Triton X-100, Sigma) were also studied. No difference in the appearance of the foods was evident between foods treated in different liquids. Tap water with chemical additives and de-aerated water produced more pronounced wave patterns compared with plain tap water alone.

Microbiological assessment of ultrasound decontamination

Small-scale decontamination of fresh produce

Small-scale washing trials were used to assess the decontamination efficiency of ultrasound (32–40 kHz) and chlorine, the current industry standard (Fig. 2). For the water dip control (without agitation), a reduction of 0.7 logs (81% reduction) of *S. typhimurium* on cut iceberg lettuce was demonstrated. Reductions of 1.6 and 1.7 logs of *S. typhimurium* (97 and 98% reduction, respectively) were observed for ultrasound or chlorine only which suggests that the decontamination efficiencies of both treatments are comparable. These log reductions were significantly different ($P < 0.05$) from the water control. In addition, when ultrasound and chlorine were used in combination, there was a further 1 log reduction of *S. typhimurium* compared with ultrasound or chlorine alone. This corresponded to a 99.8% reduction in attached bacteria.

Large-scale decontamination of fresh produce

Large-scale washing trials were used to compare the decontamination efficiency of various ultrasound and washing treatments. For statistical analysis, the washing treatments were placed into twelve different groups which corresponded to three different washing techniques (tap water, chlorine, chlorine and surfactant) with the ultrasound frequency set at four different levels (0, 25, 32–40 and 62–70 kHz). This effectively gave a four by three grid of results. With this structure the effect of the washing techniques and ultrasound frequency were evaluated. However, the treatments were investigated on different days and each batch of inoculated fresh produce had therefore to be evaluated prior to washing in order to establish the quantity of *E. coli* contaminants that had been removed. As the evaluation process was destructive, i.e. the same sample cannot be washed and then evaluated after the original evaluation has taken place, no pairing structure was applied to the data. All of this analysis was repeated on cabbage, iceberg lettuce, parsley and strawberry (Table 1a–d, respectively) (Fig. 3).

The mean of the results for each of the treatments was subtracted from the mean of the respective control to effectively give an average log reduction in attached bacteria. These values were then used to perform the final analysis. An analysis of variance (ANOVA) model was fitted to the data in order to detect which of the factors (if any) were having an effect. The four data sets from the different food products were amalgamated. The possibility of a product effect and also the possibilities of interactions were also considered.

There are some anomalies apparent in the tables. For instance the water-only treatment on the strawberry marginally increased the microbial loading (Table 1d). Although this is unlikely, there

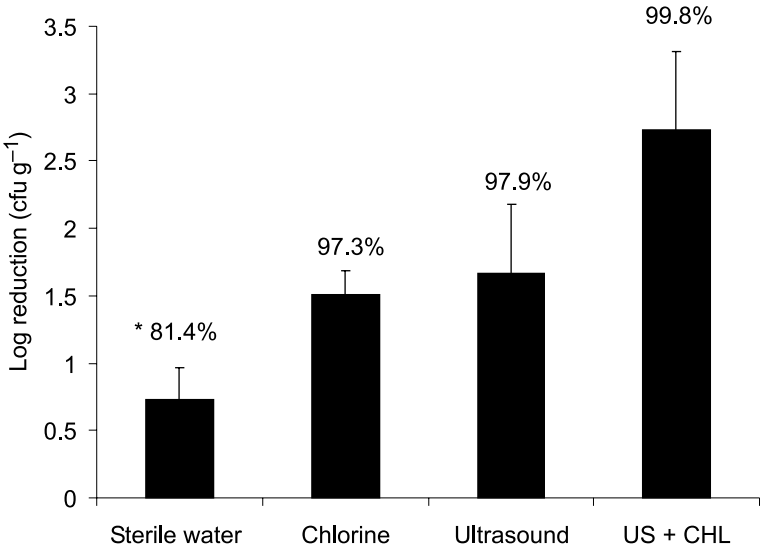

Figure 2 The effects of ultrasound and chlorine on the decontamination efficiency of *Salmonella typhimurium* on iceberg lettuce. Remaining bacteria were enumerated before and after washing to assess bacterial attachment ($n = 5$).

Table 1 (a) Large-scale cabbage decontamination trials to investigate the washing efficiency of ultrasound and chlorine treatments, (b) large-scale iceberg lettuce decontamination trials to investigate the washing efficiency of ultrasound and chlorine treatments, (c) large-scale parsley decontamination trials to investigate the washing efficiency of ultrasound and chlorine treatments, (d) large-scale strawberry decontamination trials to investigate the washing efficiency of ultrasound and chlorine treatments, (e) analysis of variance on the large-scale strawberry decontamination data

Average $\log_{10}$ reduction in attached *E. coli*

Treatment	Water only	Chlorine	Chlorine and surfactant	Mean
(a)				
Frequency (kHz)				
0	0.65	1.41	–	1.85
25	0.91	1.74	2.91	2.01
32	1.61	2.05	2.38	0.78
70	1.40	1.93	2.31	1.88
Mean	1.14	1.76	2.53	1.81
(b)				
Frequency (kHz)				
0	0.38	0.72	–	0.55
25	0.97	1.26	2.11	1.45
32	0.19	1.93	1.88	1.33
70	0.97	1.59	1.12	1.23
Mean	0.63	1.38	1.70	1.19
(c)				
Frequency (kHz)				
0	0.85	1.56	–	1.20
25	0.77	0.58	1.21	0.85
32	0.29	0.84	0.11	0.41
70	0.49	1.08	1.08	0.88
Mean	0.60	1.01	0.80	0.80
(d)				
Frequency (kHz)				
0	−0.09	2.12	–	1.02
25	1.38	1.37	1.20	1.31
32	0.77	1.58	1.96	1.44
70	0.63	1.50	1.25	1.13
Mean	0.67	1.64	1.47	1.26

(e)

Source	d.f.	Seq SS	Adj SS	Adj MS	*F*	*P*
Product	3	5.0144	5.0144	1.6715	7.82	0.000
Treatment	2	6.2133	5.6025	2.8013	13.10	0.000
KHz	3	0.3162	0.3162	0.1054	0.49	0.690
Error	35	7.4851	7.4851	0.2139		
Total	43	19.0290				

is no reason as to why this datum should be omitted from the analysis. The ANOVA model was fitted to the data (Table 1e).

The main effects of product type, treatment and ultrasound frequency on the efficiency of decontamination are summarized in Fig. 3. The different washing techniques gave a systematic difference ($P < 0.1$). The average reductions in attached *E. coli* for water, chlorine and surfactant were approximately 0.8, 1.5, and 1.6 logs, respectively. The treatments and the food products were significantly different (at the 0.1% level) from each other. A Newman-Keulls test indicated that the chlorine and surfactant treatment was significantly better than the tap water only treatment. There were no other differences between the

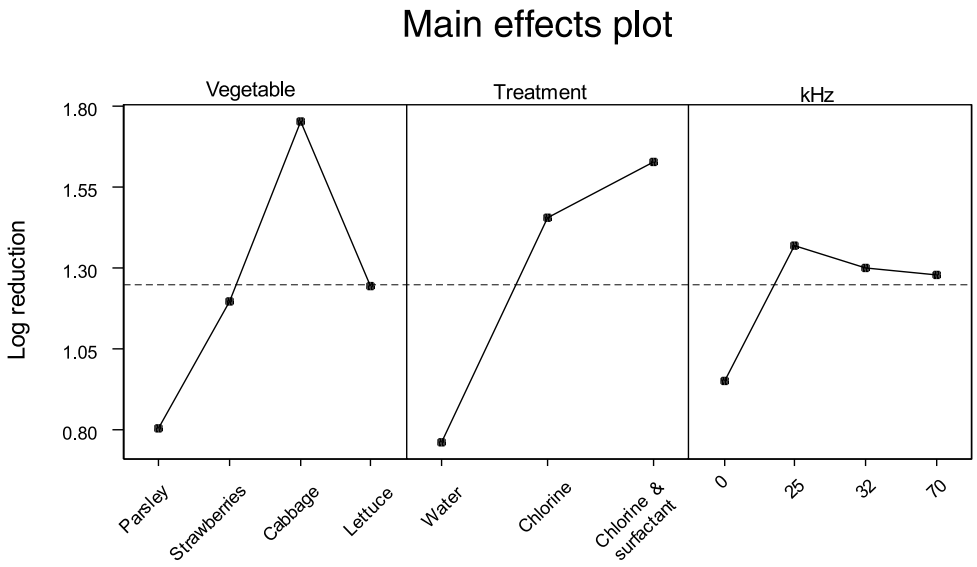

Figure 3 Summary of the effects of the main large-scale washing treatments. Dashed Line indicates the mean log reduction for all the treatments.

treatments. In terms of the differences between the product types, cabbage was the most easily washed (1.8 log reduction) and significantly differed in decontamination efficiency from the other three products. Parsley was the least easily washed, again significantly different from the other three products (0.8 log reduction).

However, the strawberry and the iceberg lettuce were not statistically different from each other (1.2 log reductions). In contrast, the frequency of ultrasound treatment had no significant effect on decontamination efficiency ($P > 0.69$). The average reductions for 25, 32–40 and 62–70 kHz treatment were 1.4, 1.3 and 1.3 logs, respectively. The no ultrasound control (0 kHz) displayed a reduction of 1.0 logs (0.3–0.4 cycles lower than US treatment).

Discussion

Bacterial attachment

The results suggest that fruits and vegetables with cut surfaces are more difficult to decontaminate than the whole uncut products (Fig. 1). The surfaces of fruits and vegetables confer protection against infection, however, pathogens can colonize plant tissue through a variety of openings. Cut surfaces, wounds and abrasions (during processing) as well as stem scars and natural openings (stomata, lenticels), provide points of entry for pathogens (Boyette *et al.*, 1993). Itoh *et al.* (1998) observed *E. coli* O157:H7 on the outer surfaces, inner tissues of cotyledons and in the stomata of radish sprouts. Lin & Wei (1997) indicated that cutting transferred *S. montevideo* onto the interior surfaces of tomatoes. Several authors have shown that the common sites for microbial aggregation of leaf surfaces are the veins, trichomes, stomata and cell wall junctions (reviewed by Carmichael *et al.*, 1999). Trimming, peeling and slicing increase the surface area for microbial attachment and provide a site of entry for microbial colonization. This work has highlighted the difficulty in removing attached microbial pathogens from both cut and uncut product surfaces.

Further research is required to better understand the mechanisms through which pathogens can contaminate MPR fruits and vegetables, either on the surface or in internal tissues.

Ultrasound decontamination of fresh produce

The operating limits of ultrasound frequency, power, time and temperature that did not lead to subjective breakdown of fruit and vegetable products were determined. Only the mint showed any notable change in appearance caused by the

ultrasound treatment in a tank. The lack of changes to other materials shows the relatively low power density of the tank treatments. Tests at higher powers using the bar applicator produced damage to mint and some other foods whilst all of the foods showed noticeable quality changes after treatment for 10 s in the high power radial applicator. The high power ultrasound systems were not progressed because of the lack of significant microbial decontamination during preliminary studies, together with the adverse effect on product quality.

Small-scale trials indicated a similar decontamination efficiency to that observed on chicken skin, where treatment at 20 kHz for 30 min in combination with chlorine gave a 2.9 log reduction of attached *Salmonella* spp. (Lillard, 1993). This is similar to the 2.7 log reduction of *S. typhimurium* on iceberg lettuce reported here. In contrast, Sams & Feria (1991) noted no significant changes in aerobic plate counts on ultrasound treated chicken drumsticks. They concluded that the irregular skin surface might have provided some level of physical protection for bacteria against cavitation. Results from our microbial attachment studies certainly suggest that this may be the case.

Attached or entrapped *Salmonella* are not readily accessible to chlorine. However, accessibility to chlorine improved when the lettuce was treated with ultrasound. Cavitation appears to detach cells, which were attached or entrapped on the surface of the lettuce, making *Salmonella* more susceptible to the sanitizer. No *Salmonella* were detected in the chlorinated wash water after a 10-min treatment. In contrast, low levels of viable *S. typhimurium* were present in the ultrasound treated wash water. No appreciable difference in pathogen viability was evident when aqueous suspensions of *L. monocytogenes*, *S. typhimurium* and *E. coli* were treated at 20 kHz for 10 min. Ordonez *et al.* (1987) commented that not all micro-organisms show the same sensitivity to cavitation. Generally, small round cells, gram-positive bacteria and spores are more resistant while protozoa and gram-negative bacteria are sensitive (Scherba *et al.*, 1991). However, Ciccolini *et al.* (1997) showed that cavitation at nonlethal temperatures had little effect on the viability of *Saccharomyces cerevisiae*. It is therefore not surprising that no significant differences in viability were evident in experiments after 10 min. Bacteria detached from the surfaces of fruits and vegetables by cavitation will be released into the wash water and may have the potential to cross-contaminate. Ultrasound should be used in combination with disinfectants in the wash water. In these investigations, the chlorinated wash treatments were significantly better than the water alone (at the 0.1% level). However, although chlorine and surfactant (dioctyl sodium sulphosuccinate) demonstrated an increased 0.2 log washing efficiency compared with chlorine alone, this difference was not statistically significant. Dewhurst *et al.* (1986) observed an improved recovery of *Bacillus subtilis* spores using a surfactant (Tween 80). Nonetheless, Adams *et al.* (1989) indicated that chlorine and Tween 80 in combination were no more effective at decontaminating lettuce than Tween 80 alone. In fact, the surfactant exerted a chlorine demand on the system and reduced the available chlorine by 30%.

Cavitation enhances the mechanical removal of attached or entrapped bacteria on the surfaces of fresh produce by displacing or loosening particles through a shearing or scrubbing action.

Throughout treatment, microsteaming of gas bubbles and strong eddies could be seen, which is typical of stable cavitation.

The mechanical cleaning efficiency of cavitation in the large-scale trials was significantly lower than that for the small-scale experiments, for a number of possible reasons. Untreated potable (tap) water was used throughout all the large-scale trials to mirror industrial practice. However, the small-scale trials used completely degassed autoclaved tap water. There was a tendency for water impurities, for example calcium salts, to precipitate out of solution during sterilization. Water hardness and dissolved gases can significantly reduce cavitation efficiency. Degassing and removal of water hardness agents could be a potentially expensive process, and are possible limitations on industrial implementation.

With the potentially high capital expenditure for equipment and water treatment, it is unlikely that the fresh produce industry will be willing to adopt this technology. Furthermore, the additional one log reduction achieved by applying ultrasound to a chlorinated water wash does not completely eliminate the risk of pathogens on fresh produce.

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