Project summary VM20007

Effective control of *Listeria* on rockmelons through alternative postharvest treatment methods

Hort Innovation MELON FUND

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Effective control of *Listeria* on rockmelons through alternative postharvest treatment methods

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LISTERIA MONOCYTOGENES: A CAUSE OF SERIOUS DISEASE

Food safety is a priority for all horticultural industries. This is particularly the case for crops where the harvested part is grown in or near the soil and may be eaten uncooked.

KEY POINTS

- *Listeria monocytogenes* can potentially contaminate rockmelons during production or postharvest handling
- Bacterial populations can increase rapidly if *Listeria* on the rind transfer to the flesh during preparation and storage, even under refrigerated conditions
- *Listeria* are hard to remove due to the complex structure of the rockmelon rind as well as their ability to form protective 'biofilms' within hours of contamination occurring
- Trials were conducted using a washing plus brushing unit which simulated the run-to-waste systems used on commercial packing lines
- Increasing the sanitiser concentration above normal rates did not improve effectiveness against *Listeria* inoculated onto the rind 24 hours before washing
- Increasing dwell time from one to four minutes increased removal of *Listeria* from the rind approximately 100-fold, reducing the initial population by 3-log or more
- Although differences between sanitisers were frequently not significant, there was a consistent trend to PAA being more effective against *Listeria* than chlorine (NaOCl)
- The effectiveness of washing plus brushing with sanitiser was not reduced when melons were dirty
- The washing plus brushing system is highly effective at removing / killing bacteria due to the mechanical action of the brushes, which disrupt the *Listeria* biofilm
- Although it is difficult to completely clean bacteria from brushes, the advantages of washing plus brushing are likely to outweigh the potential risks from cross contamination
- Where a biofilm is not present (e.g. cross contamination during washing), *Listeria* populations are more easily removed, so even a short rinse in water can significantly reduce the population

The key bacteria responsible for food safety outbreaks include Shiga-toxin producing *E. coli* (STEC), *Salmonella* spp. and *Listeria monocytogenes* (*Lm*). Of these, *Lm* is the hardest to control.

Listeria are soil dwelling bacteria commonly found in farm environments. *Lm* can live in mammals, frogs and birds as well as rotting vegetation, manure, soil and water. It can grow at temperatures ranging from -1.5°C to approximately 45°C, at pH levels from 4.0 to 9.5, and can survive freezing and even immersion in a 14% salt solution.

In 2019 NSW DPI, together with the Australian melon industry and Hort Innovation, published 'Melon food safety: A best practice guide for rockmelons and specialty melons'[1]. The full guide can be accessed **here** or by scanning the QR code below.

This project adds to the previous research but with a specific focus on *Listeria*. Removal of *Listeria* from rockmelon skin is particularly challenging due to the formation of sanitiser resistant biofilms. This report details the effectiveness of different practices at breaking down this biofilm, with the target of achieving a 3-log (99.9%) reduction in *Listeria* populations on rockmelon rind.

WHY IS *L. MONOCYTOGENES* SUCH A PROBLEM FOR MELONS?

It comes down to the skin. The netted skin of rockmelons is full of cracks and pores for bacteria to hide in. This makes removal particularly difficult. Rockmelons are one of the few products where *Lm* can survive, and even grow, on the skin after harvest.

Even though the skin is not eaten, bacteria can transfer into the flesh during preparation. If cut melon is not eaten immediately, the sweet, wet, low acid environment the flesh provides allows the bacteria to multiply, even under refrigerated conditions.

Sanitisers are chiefly used to prevent cross contamination in wash water. They are not as effective at removing bacteria already on fresh produce. Despite this limitation, sanitisers are still the best way to reduce the risk of bacteria from the field surviving the packing process.

Listeria are particularly difficult to remove as they can form protective 'biofilms' within hours of contamination occurring. These are resistant to chemical sanitisers, adding to the difficulty of control[2].

This guide outlines what we know about the most effective way to kill or remove *Listeria* from rockmelon skin, reducing the risk of contaminated products entering the supply chain.

SANITATION METHODS CURRENTLY USED BY INDUSTRY

While the time between harvest and sanitation varies, many practices are now standard through the industry. Following a dry bin tip, melons are cleaned using a series of rotating brushes. Some farms even have a double layer, with melons brushed from above as well as below.

As the melons move through the line, nozzles over the brushes spray them with sanitiser. Some farms apply a fine mist, others use lower pressure and coarse jets to create large droplets. In all farms surveyed, sanitiser was applied as run-to-waste, limiting opportunities for cross contamination.

Dwell time through brushing and sanitation is quite variable. It may be as little as 40 seconds to several minutes, depending on how many melons are entering the line, and the system used to move them forward.

Sanitation is generally followed by drying and postharvest disease control. This may take the form of a short heat treatment, or fungicide application.

TYPES AND CONCENTRATIONS OF SANITISERS USED

Industry currently uses a range of sanitisers, including sodium hypochlorite, chlorine dioxide and mixtures of peroxyacetic acid (PAA) and hydrogen peroxide. Other options include calcium hypochlorite (granules), bromo-chloro-dimethyl hydantoin (Nylate®), e-water and even citric acid. Ozone can be used to sanitise water, but has no residual effect on the melon surface.

Sanitisers are frequently applied at high concentrations, especially if the melons are dirty. For example,

- Chlorine can be effective against microbes in water at 10ppm or less
- Other fruit and vegetables are commonly washed with water containing 100ppm chlorine
- Many growers use 200ppm chlorine during washing of melons
- The chlorine concentration may be increased to as high as 300ppm if melons are dirty.

Although increasing the sanitiser concentration seems a straightforward way to improve efficacy, such increases are not without cost. Chlorine is classified as a hazardous chemical. Exposure to fumes can irritate the nose, throat and eyes of workers. At high levels, chlorine can result in difficulty breathing, skin injuries, chest tightness and severe eye irritation.

Chlorine sanitisers are corrosive, potentially damaging equipment, especially rubber and plastic fittings. Other products, such as PAA, are relatively expensive. There is also the issue of disposal of wastewater; water containing sanitiser has to be disposed of safely and with care for the environment, which is more difficult with high concentrations.

The best system is one which applies enough sanitiser to achieve the target reduction in bacteria, but without using excessive, wasteful or damaging concentrations.

Calcium hypochlorite

Sanitisers can vary in their effectiveness against *L. monocytogenes*. A recent meta-analysis of the effects of different sanitisers[3] from 84 publications ranked them as follows:

1. Calcium hypochlorite (chlorine granules)
2. E-water
3. Chlorine dioxide solution
4. Peracetic acid (PAA)
5. Sodium hypochlorite (liquid chlorine)
6. Citric acid

Chlorine granules are difficult to use in commercial spray systems due to the likelihood of blocking the nozzles. E-water is relatively new but has already been adopted by some processors of leafy greens, but the setup cost of systems is still relatively high.

FINDING WAYS TO REMOVE *LISTERIA*

We have tested how well different sanitisers remove *Listeria* artificially inoculated onto the rind of rockmelons. In these trials we used *Listeria innocua* (*Li*), which is almost genetically identical to *Lm* but not dangerous to humans. We confirmed the suitability of this strain of *Li* as a surrogate for *Lm* by comparing its susceptibility to different concentrations of common sanitisers.

The melons were inoculated 24 hours before washing, then allowed to establish on the rind at 20°C and 90% relative humidity (Figure 1). This allows the *Li* to form a protective biofilm before washing, as would occur if infection occurred in the field.

The melons were cleaned for two minutes using a laboratory scale washing plus brushing unit analogous to systems used in commercial packhouses (Figure 2). They were allowed to dry, then sections of rind were removed and submitted for testing by a NATA accredited laboratory.

Populations are recorded as log values to the power of 10. A two-log reduction means that 99% of the bacteria were killed or removed, while a three-log reduction means 99.9% of the population has been killed or removed.

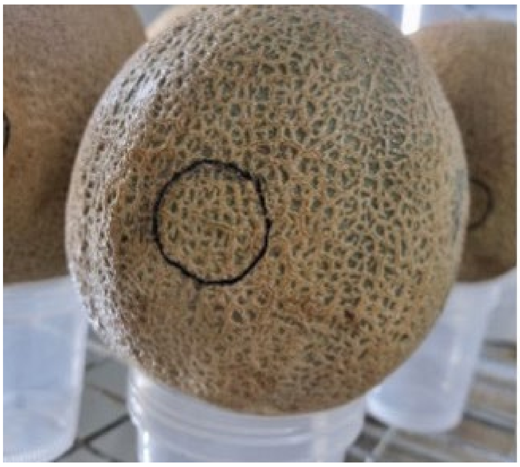


Figure 1. *Melons were artificially inoculated with Listeria bacteria, then stored overnight at 20°C and 90% RH before washing.*

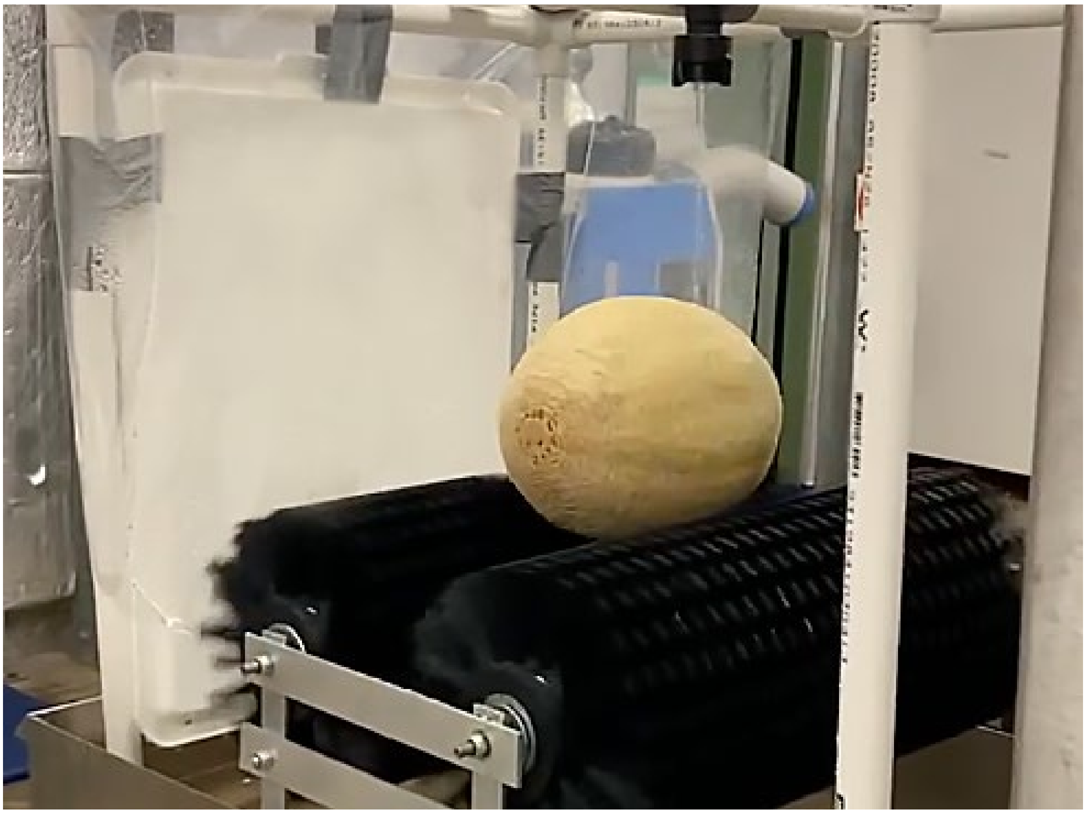


Figure 2. *Laboratory scale washing plus brushing unit, used to sanitise one melon at a time.*

EFFECTS OF SANITISER CONCENTRATION

Apart from the lowest concentration of chlorine (25ppm), effectiveness was the same regardless of sanitiser concentration. This is less surprising than it might seem; other researchers have also found that the concentration of sanitiser did not make a significant difference to the results[2,4].

In this trial all of the sanitisers reduced the population of *Listeria* on melon skin by 2.5 to 3.2 logs (Figure 3).

The lack of differences between concentrations, as well as the high log reductions achieved, suggest that most bacteria were removed by the mechanical action of brushing, rather than through chemical action of the sanitisers.

Previous researchers have also found that mechanical removal is effective. For example, Fu et al[5] demonstrated that abrasive brushing was effective at removing *Listeria* biofilms from melons.

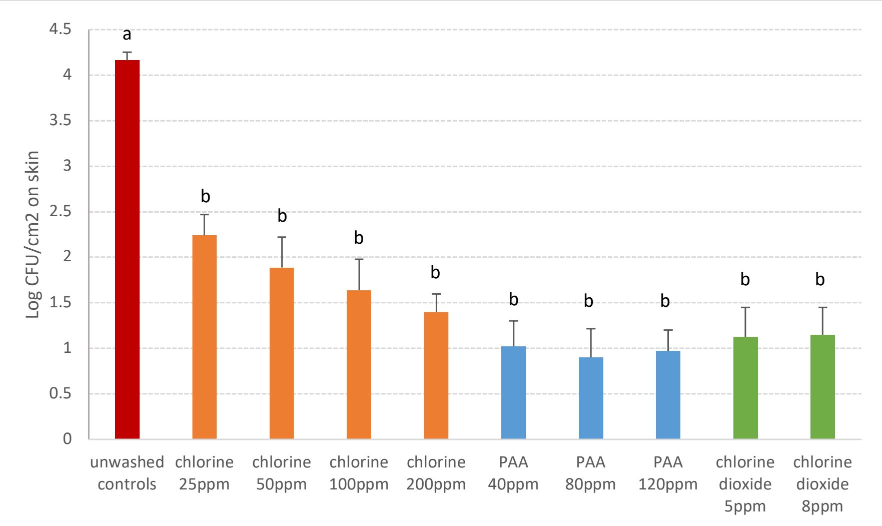


Figure 3. *The effect of 2 minutes washing + brushing with different sanitisers, applied at a range of concentrations, on removal of L. monocytogenes from melon rind*

HOW LONG SHOULD MELONS BE SANITISED FOR?

The short answer is, **as long as possible**.

In our trials, increasing the dwell time during washing plus brushing significantly improved removal of *Li* from melon rind, regardless of the sanitiser applied. The exception was PAA, which was as effective when melons were brushed for one minute as when they were brushed for two (Figure 4).

Due to issues with the e-water generator, in this trial we substituted tap water – and achieved the same result. This confirms that the brushing action is more critical for removing *Listeria* than the sanitiser used.

Note that 2 minutes brushing resulted in 2.5 to 3.2 log reductions in the previous trial, but 1.6 to 2.7 log reductions here. The reasons for this difference are unclear. However, the key message is that each doubling of dwell time increased removal of *Listeria* by approximately 10-fold. Maximizing dwell time is essential.

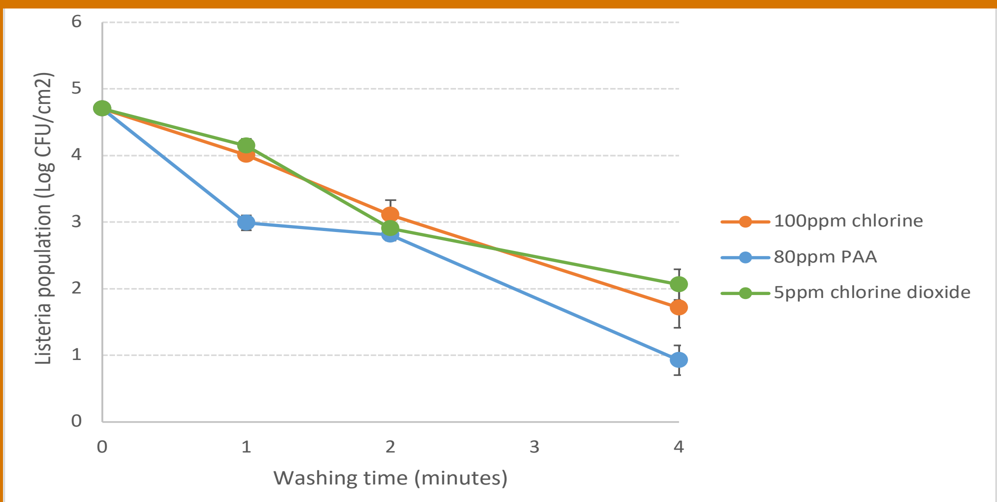


Figure 4*. The effect of washing + brushing dwell time with different sanitisers on removal of L. monocytogenes from melon rind*

WHAT IF THE MELONS ARE DIRTY?

Sanitisers react with organic compounds, including soil. Chlorine, in particular, is rapidly deactivated if the water becomes dirty. Dirty water is a major issue in recirculating systems. However, the melon sanitation systems used by industry are run to waste, so this is may be less of an issue.

To test this, we added *Li* to sterile clay loam soil, turned the soil into a wet slurry, then used this mixture to inoculate the melons. A matched set of melons was kept clean and inoculated as previously. All were allowed to incubate at 20°C for 24 hours then washed plus brushed in tap water, 100ppm chlorine, 80ppm PAA or 5ppm chlorine dioxide.

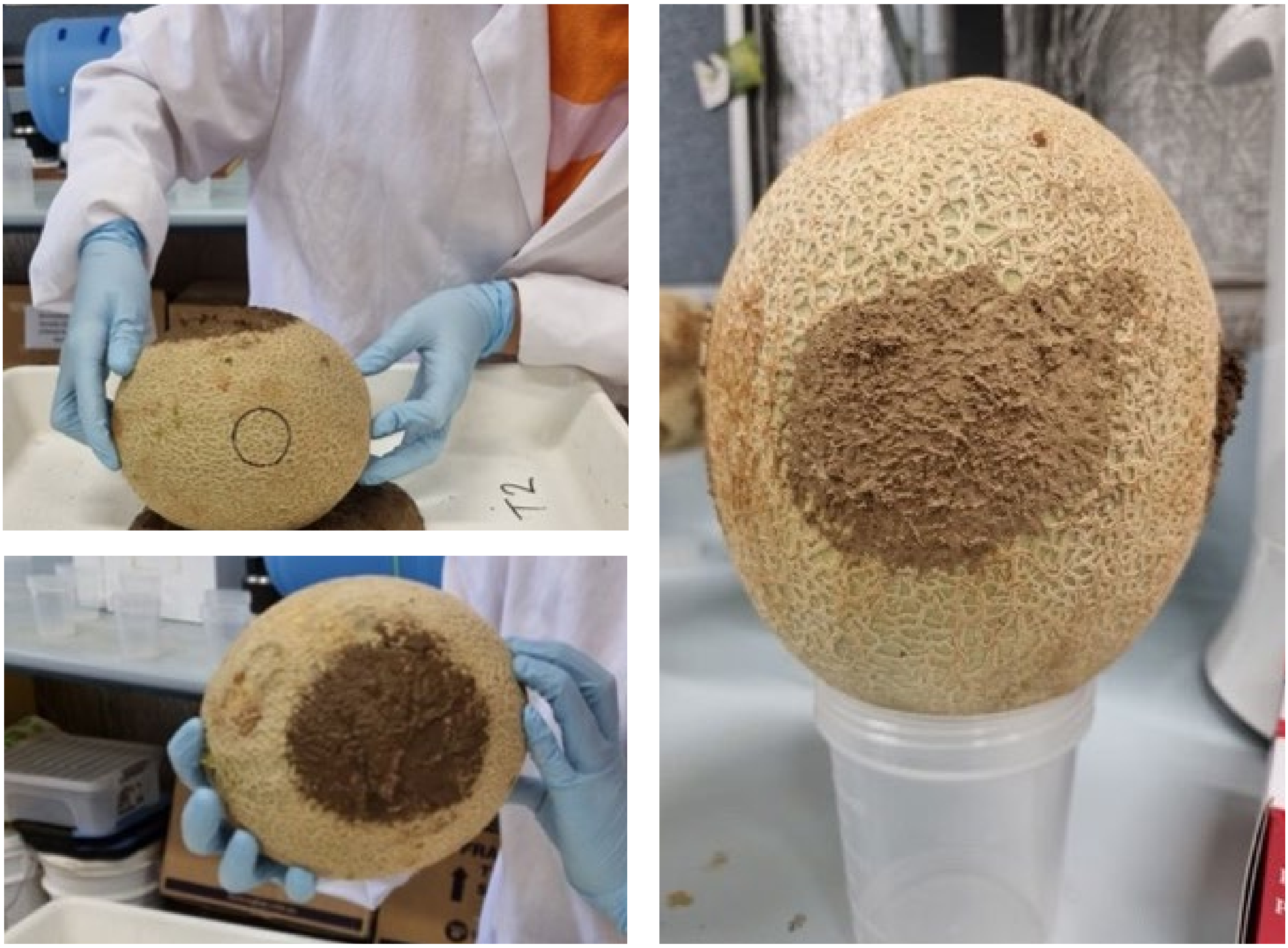
All of the unwashed controls were positive for *Li*. However, in this trial, *Li* was below detectable levels on many of the washed melons.

Given the number of 'zeros', it is more useful to consider the number of melons which remained positive for *Li*, than log reductions achieved.

The presence of dirt did not increase detections, but actually made it slightly easier to remove the adhering *Li* it contained. Just over half the melons brushed for two minutes with tap water did not have detectable populations of *Listeria* (Figure 6). Adding chlorine or chlorine dioxide further reduced the number of detections.

In this trial PAA was completely effective, reducing *Li* below detectable levels on all cleaned melons regardless of the contamination method used.

Figure 5. *Melons were inoculated using a soil slurry containing approximately 6 log CFU/g Listeria, then allowed to dry for 24 hours before washing plus brushing.*



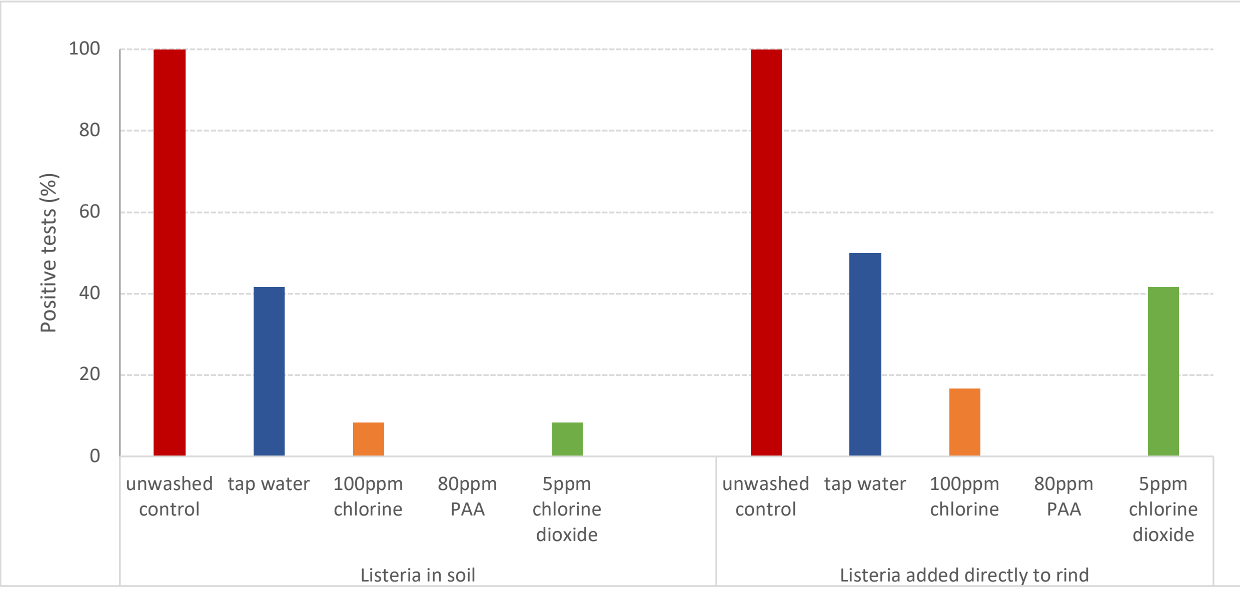


Figure 6. *Proportion of melons (n=12) which still had detectable populations of Listeria after 2 minutes washing plus brushing in tap water, 100ppm chlorine, 80ppm PAA or 5ppm chlorine dioxide. Melons were inoculated by dipping in contaminated soil or direct inoculation of culture onto the rind. Numbers above each column indicate the average population of Listeria on positive samples only (log CFU/cm^2).*

WHAT ABOUT CROSS CONTAMINATION FROM THE BRUSHES?

Contamination of brushes is a potential issue that must be considered.

Li was detected on the rind when we ran clean melons through the brushes after contaminated ones. The bacteria could persist and transfer onto clean melons even if a sanitiser was used or the brushes were dried then rinsed with 70% ethanol.

However, our laboratory based work represents a worst-case scenario. The brushes contacted very high populations of *Li* at the same position, multiple times, with no final rinse. This is unlikely to occur commercially.

While it is true that brushes are difficult to clean, it seemed likely that the benefits from brushing would outweigh potential risks.

To verify this, we compared the effect of simply dipping melons into a sanitizing solution instead of brushing them. The melons were inoculated onto the rind and incubated for 24 hours as previously.

One minute in 60°C water can help reduce postharvest disease. It seemed possible this could enhance removal of bacteria as well, even without brushing. The treatments used were therefore either dipping or brushing for one minute with tap water, hot (60°C) tap water or 100ppm chlorine.

Dipping in tap water, whether ambient or 60°C, failed to significantly reduce *Li* populations. In contrast, washing plus brushing for 1 minute removed 1 log (90%) of *Li*, consistent with the results in Figure 4. In this trial adding chlorine increased removal of *Listeria* 10-fold (Figure 7), whereas previously it had no significant effect, another example of the variability in microbial results.

However, mechanical brushing consistently removed *Li* in all trials. Mechanical brushing is effective because it

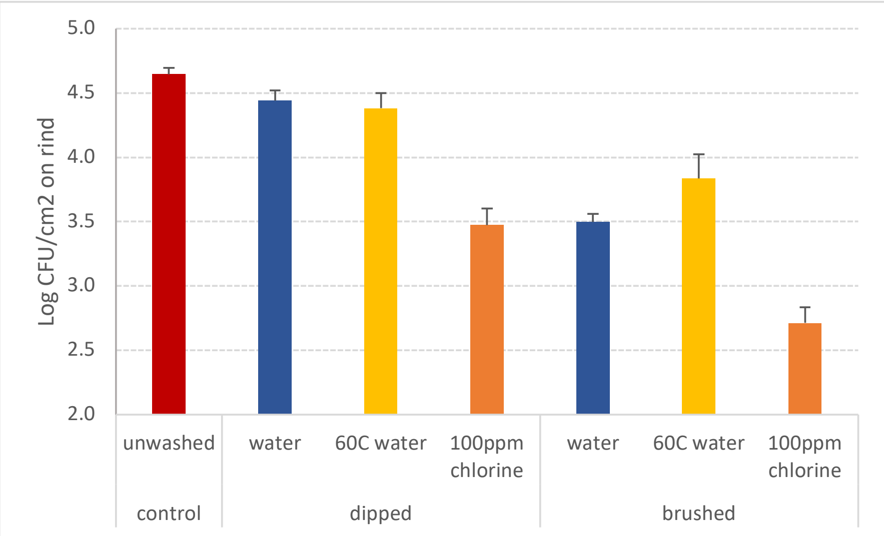


Figure 7. *The effect of dipping compared to washing plus brushing for 1 minute on removal of Li from melon rind using tap water, 60°C tap water or 100ppm chlorine.*

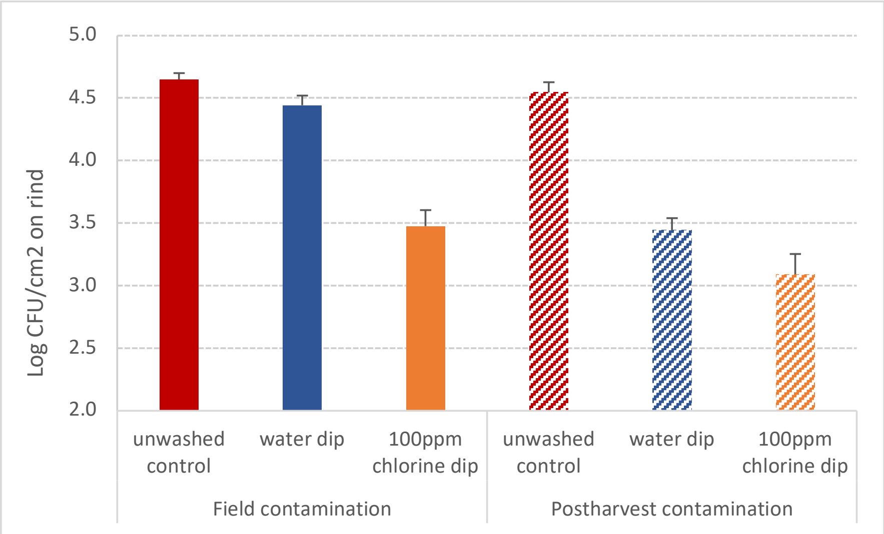


Figure 8. *The effect of a one minute immersion in tap water or 100ppm chlorine on removal of Li from melon rind when melons were inoculated 24 hours (field contamination) or 1 hour (postharvest contamination) before washing*

breaks through the protective biofilm that *Listeria* can form within hours of infection. In contrast, bacteria that are accidentally transferred to the rind postharvest will be much easier to remove.

We confirmed this by comparing how many *Li* were killed or removed when *Li* was inoculated onto melon rind either one hour or 24 hours beforehand.

Although dipping in tap water was ineffective at removing bacteria inoculated 24 hours prior, it removed 90% of bacteria transferred one hour before rinsing. The delay between inoculation and washing was less important when chlorine was included. However, 100ppm chlorine solution was significantly more effective when used against 'postharvest contaminant' bacteria than those inoculated 24 hours before.

A final rinse may therefore be effective at removing any *Listeria* that have cross-contaminated melons postharvest.

This result suggests that if a long series of brushes are used to provide the necessary dwell time, then any *Listeria* that transfer from contaminated brushes at the start of the line are likely to be removed by the end.

BEST PRACTICE TO MANAGE THE RISK OF LISTERIA CONTAMINATING MELONS

- ✓ Wash melons using series of rotating brushes for a **minimum** of two minutes, longer if possible
    - Use a movement bar to ensure minimum wash times are met
    - Wash times of 1 minute or less will be much less effective
    - Spraying or dipping in sanitiser without brushing will not be effective
- ✓ Wash water should contain 80ppm PAA (preferred), 100ppm chlorine or 5ppm chlorine dioxide
- ✓ Apply wash water + sanitiser liberally, ensuring the whole melons is well coated, and allowed to run-to-waste
- ✓ Clean and sanitise brushes regularly (e.g. daily if possible)
    - Physically clean to remove built up dirt or juice
    - Steam or boiling water is the best way to sanitise brushes
    - 70% ethanol or a food grade quaternary ammonium compound may also be used
- ✓ If melons are dirty, increase the washing time

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