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Technical Report: The effective control of *Listeria* on whole rockmelons through alternative post-harvest treatment methods

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Abbreviations and acronyms

AIDS	Acquired immunodeficiency syndrome
ALOP	Acceptable level of protection
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists International
APVMA	Australian Pesticides and Veterinary Medicines Authority
ARC	Australian Research Council
ATP	Adenosine triphosphate
BHIB	Brain Heart Infusion Broth
BOD	Biological oxygen demand
CDC	(USA) Centers for Disease Control and Prevention
CFU	Colony forming unit
CLL	Chronic lymphocytic leukaemia
CO	Cinnamon oil
COD	Chemical oxygen demand
DBP	Disinfection by products
DNA	Deoxyribonucleic acid
ECGA	Eastern Cantaloupes Growers Association (USA)
EDTA	Ethylenediaminetetraacetic acid
EHEC	Enterohemorrhagic *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
FDA	United States Food and Drug Administration
FSANZ	Food Standards Australia New Zealand
FSO	Food Safety Objective
GA	Gum arabic
GAP	Good Agricultural Practice
GHP	Good Hygienic Practice
GMP	Good Manufacturing Practices
GRAS	Generally Recognized As Safe
HACCP	Hazard Analysis and Critical Control Points
HD	Honeydew melon
HIV	Human Immunodeficiency Viruses

HPLNC	A specific sanitiser including hydrogen peroxide, sodium lactate, nisin and citric acid
ICMSF	The International Commission on Microbiological Specifications for Foods
IIA	International Irradiation Association
LAE	Lauric arginate
LED	Light emitting diodes
LOD	Limit of detection
LVA	Levulinic acid
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
MPN	Most Probable Number
NR	Not Reported
OH&S	Occupational Health and Safety
ORP	Oxidation-reduction potential
PAA	Peroxyacetic acid
PMA	Produce Marketing Association
RH	Relative Humidity
SD	Standard Deviation
SDS	Sodium dodecyl sulfate
SHS	Superheated steam
SO	Soybean oil
SS	Saturated Steam
STEC	Shiga toxin producing *Escherichia coli*
TSS	Total soluble solids
TVC	Total viable count
UV	Ultraviolet light
VSV	Vacuum/steam/Vacuum
WG	Western Growers (of cantaloupes in USA)
WGS	Whole Genome Sequencing
WHO	World Health Organisation
WM	Watermelon
YOPI	Young, Old, Pregnant, Immunocompromised

Executive Summary

Introduction

The Australian Melon Industry is one of the larger Australian fruit industries. Major production regions include Queensland (Bowen, Bundaberg and Chinchilla), New South Wales (Cowra and Riverina), the Northern Territory (Darwin and Katherine) and Western Australia (South Perth, Carnarvon and Kununurra). In 2017/18, 215,519 tonnes of melons were grown, valued at $124.2 million, of which rockmelons made up 25%.

The bacterium *Listeria monocytogenes* has previously caused sporadic cases of the disease listeriosis linked to rockmelons, but a large outbreak that occurred in Australia in early 2018 resulted in 22 cases, 7 deaths, and a miscarriage. A smaller outbreak of 9 cases and 2 deaths occurred in 2010. The consequences of the 2018 outbreak were felt by both consumers and the entire industry through illness, loss of life, and loss of sales and livelihoods in the industry. In the Australian 2018 outbreak, residual, low-level, contamination on the rind of whole melons but with high prevalence was considered to have been a risk factor for human illness.

Despite that listeriosis is a rare disease, outbreaks of foodborne listeriosis from susceptible foods are not uncommon and often result in the death of consumers, or miscarriages. As noted, this can have devasting effects for the businesses or industries involved. In general, listeriosis affects people who are very young, or very old, pregnant or having a medical condition or therapy that compromises the immune system; the so-called 'YOPI' group.

Compounding the current threat from listeriosis to the food industry, including the rockmelon industry, are three factors. Firstly, that as a food business becomes larger, even if they have excellent hygiene and even if fruit is only sporadically contaminated with *L. monocytogenes* more people will be exposed, and some may become ill, require medical attention and be recognised by epidemiology networks. Secondly, modern epidemiological tools (e.g., 'real-time' national and international foodborne illness surveillance networks and, in particular, Whole Genome Sequencing technologies) mean that outbreaks from a common source can be identified across geographic regions and across time, even if only a handful of people are affected. Thirdly, the proportion of the population in developed countries that have compromised immune systems is

increasing, mostly because we are living longer, but also because the number of people living with illnesses or receiving therapies that reduce their immunity is increasing through advances in medical technology. Dietary advice to the YOPI group may be part of the risk management but can also reduce consumer confidence; currently such advice is mainly targeted towards pregnant women (about 1% of the Australian population at any given time).

In response to the risk of listeriosis, the melon industry has made further food safety research and development and minimisation of risks, through new knowledge and adoption, a top priority. Hort Innovation, using the melon research and development levy and contributions from the Australian Government, funded this 'desk-top-based' scoping study involving a team with diverse specialist expertise to i) review the learnings from outbreaks of listeriosis from melons with regards to post-harvest treatment methods; ii) review international best-practice and, from published literature, evaluate existing and alternative methods for sanitation of rockmelons that could be adopted by the Australian rockmelon industry; iii) outline recommendations for further research or adoption of international best practices in Australia to minimise the risk of listeriosis from rockmelons.

Research questions

After discussion and consideration by the research team, specific research questions were defined for the scoping study, and endorsed by Hort Innovation, to address the overarching objectives of Hort Innovation for food safety in the melon industry.

For the review of previous outbreaks, the research questions and scope were to:

1. Identify the most recent (2010 – present) confirmed or potential outbreaks that have occurred from contamination of rockmelon/melons by *L. monocytogenes* and *Salmonella* in Australia, USA, or Europe.

2. Articulate the key findings regarding the route of *L. monocytogenes* contamination of rockmelons, and whether there are similarities or differences across the rockmelon outbreaks identified.

3. Identify the key outcomes or recommendations that arose following the investigations of the identified outbreaks.

The research questions and scope for the reviews of best practice recommendations, primary production interventions, growth rates, prevalence, and internalisation were defined as:

1. What 'best practice recommendations' have previously been provided to the rockmelon industry, either in Australia or internationally, to reduce the prevalence of *L. monocytogenes* on rockmelons (from primary production to packaging and transport)?

2. What research exists describing the effectiveness of different interventions to minimise/control of *L. monocytogenes* on whole melons at all stages from primary production to when melons leave the farm gate? What emerging technologies may be applicable, what data gaps exist, and where is further useful research required?

3. What research exists regarding growth rates, prevalence, or internalisation of *L. monocytogenes* in/on rockmelons from primary production to consumption. What data gaps exist and where is further research required?

Methods

Before undertaking the literature review and analysis, to provide context and to understand the growing and processing conditions in the Australian rockmelon industry, we visited farms and packhouses in eastern Australia (NSW and Far North Queensland), and Western Australia (Carnarvon and Perth region), and spoke with growers/processors and their technical staff to understand their needs and their experiences regarding practices that affect rockmelon food safety. This also provided team members with additional understanding to be able to better assess the relevance of the literature we found to Australian rockmelon industry conditions.

The research team met to determine the scope, research questions and key themes for the literature review process that would address the project objectives. Literature search strategies were designed to identify all publications relevant to the project objectives. The search strings (both search terms and Boolean operators) were documented, and two high-coverage scientific databases (Web of Science and Scopus) were searched. Grey literature, including industry and regulatory websites, and books, and Google Scholar were also searched. Experts and industry stakeholders were consulted and invited to identify relevant literature. The reference lists of

articles identified as relevant were searched for additional relevant publications. Relevant literature was collated, and then de-duplicated.

Quantitative data describing reductions in pathogens due to interventions was extracted from tables in publications or derived from graphs using the online tool WebPlotDigitiser. All information included in the review was critically assessed by the research team who provided further feedback, advice, and commentary. The available data was critically assessed for reliability and 'representativeness' and this is included in the report.

Searches were undertaken from October 1st 2019 to December 30th 2019. A total of 1181 publications were initially identified and, following de-duplication, relevance screening, and characterization, 87 papers were retained and included in the review. The publication dates of these papers ranged from 2002 to 2019 (noting that the first reported listeriosis outbreak from melons occurred in 2010). All data sources and sources of other information used are fully documented in the report.

Conclusions

Learnings from outbreak investigations

L. monocytogenes is common in natural environments, particularly if rotting vegetation is available, and may occasionally be present in the faeces of humans and domestic animals. It can colonise food processing plants and is well known to present risks to businesses processing food that supports the growth of *L. monocytogenes* and that are eaten without further cooking.

Listeriosis outbreaks from whole rockmelons are rare: there are only three listeriosis outbreaks from whole rockmelons reported in the international literature in over 40 years, but all resulted in fatalities. Two of those outbreaks occurred in Australia, and one in North America.

Investigations after the two largest outbreaks suggested that high frequency contamination of rockmelons with *L. monocytogenes* contributed to those outbreaks. It was suggested that the contamination of the melons probably occurred in the packhouse after colonisation of the packhouse potentially by:

- *L. monocytogenes* on fruit after adverse weather events (heavy rainfall in December prior to harvest, followed by dust storms) (Australia 2018 outbreak); or
- introduction to the plant of contaminated 're-purposed' food processing equipment from another produce processing business (USA 2011 outbreak); or
- contamination from trucks that were transporting un-saleable melons as feed to a cattle farm (USA 2011 outbreak); or
- failure to use sanitiser spray on melons (USA 2011 outbreak);

or that high prevalence, but low level, contamination occurred in the field after adverse weather events and was not eliminated during processing in the packhouse (Australia 2018 outbreak).

Listeriosis is a rare disease but specifically affects people with compromised immune systems. Even in those people the doses required to cause infection are usually relatively high, suggesting that growth on, or in, foods contributes significantly to the likelihood of infection. *L. monocytogenes* can grow at refrigeration temperatures and can grow on the surface and in the flesh of rockmelons: while the rind is not eaten, transfer of *L. monocytogenes* onto the fruit pulp from the surfaces of the rockmelon during cutting represents a food safety risk. These characteristics mean that risk management of rockmelons from *L. monocytogenes* will require a whole of supply-chain approach, e.g., involving adoption of GAP, GMP and food safety plans/HACCP, potentially including environmental monitoring, and *actions both to minimise contamination of the fruit and to minimise the potential for growth on the fruit.*

Whole Melon Sanitisation

In response to industry requests for information on sanitiser efficacy, while it is clear that sanitisers make an important contribution to product safety, our literature review and analysis revealed limited consistent evidence to determine the efficacy of sanitisers currently used in Australia specifically to kill or remove *L. monocytogenes* on the surface of whole rockmelons (*see* Section 8). In response to specific industry queries, there is no evidence that sanitisation treatments currently used by the Australian rockmelon industry will reliably achieve > 3 $\log_{10}$CFU reductions of *L. monocytogenes* on the surface of whole rockmelons.

The 'bacterial kill' achieved by a sanitiser depends on factors such as the type of sanitiser itself, pH, temperature, organic matter, the commodity, and the target organism. In routine operation, concentration and contact time with the fruit are fundamental to sanitiser efficacy. We concluded that there is insufficient research regarding both product quality and safety to specify recommendations for optimal contact times, specifically to kill *L. monocytogenes* on the surface of melons, for currently used sanitisers. Nonetheless, in the absence of more evidence the results support the recommendations of NSW DPI (2019b) for chlorine (100ppm), peroxyacetic acid (80ppm), and chlorine dioxide (aqueous) (5ppm) contact times of 2 minutes. However, due to the potential limited efficacy of those sanitisers, as demonstrated in this scoping study, rockmelon food safety management will also require the consistent implementation of a whole-chain approach.

We identified and summarised research concerning a number of potential alternative sanitisation methods in response to industry desire to identify potential sanitisers/systems that can produce > 3 $\log_{10}$CFU reductions in *L. monocytogenes* on the surface of whole rockmelons. Several promising technologies (including, but not limited to, X-rays, octenidine dihydrochloride, hot water, superheated steam, and dry steam) have been investigated and reported to produce > 3 $\log_{10}$CFU reductions in *L. monocytogenes* on the surface of whole melons or rind sections. However, due to limited research, cost, practicality, and other considerations, not all of these will be relevant for the Australian industry. Determination of cost/benefit for these proposed treatments was beyond the scope of this review. However, we have provided general indications of the potential benefits and limitations in this report for all sanitisers/treatments.

Recommendations

Whole-of-chain Risk Management

The previous publications "Melon Food Safety: A Best Practice Guide for Rockmelons and Specialty Melons" (NSW DPI, 2019b) and "Melon food safety toolbox: Practical resources for implementing best practice"(NSW DPI, 2019a), prepared by Dr Sukhvinder Pal (SP) Singh from NSW Department of Primary Industries, represent the most relevant and recent comprehensive advice provided to the Australian melon industry and should be reviewed by all stakeholders.

In relation to minimisation of the risk of *L. monocytogenes* from Australian rockmelons we recommend further research should:

- further develop and communicate a holistic risk management strategy that includes growers assessing and responding to adverse weather events, or other unusual circumstances, and more effective and reliable hygienic handling of fruit from the field and during processing and transport

- determine the prevalence of *Listeria* spp. or *L. monocytogenes* on whole rockmelons and in environmental samples, relevant to risk, at different points in Australian rockmelon supply chains and from different geographic regions. While this is being undertaken in some parts of the industry, it would be beneficial for a database to be established where results can be collated by state, and nationally, to be able to demonstrate with confidence to risk assessors and consumers the currently apparent low prevalence of *L. monocytogenes* on rockmelons and in rockmelon growing sites in Australia

- investigate the potential for internalisation of *L. monocytogenes* into whole rockmelons at different points in the rockmelon supply chain (e.g., field, packhouse, consumer handling)

- assess the potential influence of weather events on the prevalence of *Listeria* spp. on/in fruit in the field and the growing environment and the potential persistence of *Listeria* spp. both in the soil and on whole melons in the field under different weather conditions. This assessment should include collaboration with farmers/producers regarding current practices to help frame science-based risk management decisions regarding harvest after 'adverse' weather events

- further investigate the ability of *L. monocytogenes* to colonise rockmelon packhouses from environmental sources or contaminated fruit

- improve quantitative knowledge of factors, such as temperature, surface moisture, relative humidity, extent of netting, or others, that influence the potential for growth of *L. monocytogenes* on rockmelons and how those factors vary throughout the supply chain

- investigate whether regular "in-house" environmental monitoring (both factory and growing environment) is feasible and will reduce listeriosis risk from rockmelons, and if so, develop specific guidance on environmental testing programs including methods, sites, and frequencies.

Whole Melon Sanitisation

To optimise the application of sanitisers on whole rockmelons as part of a whole-of-supply-chain approach to minimise the risk of listeriosis from Australian rockmelons we recommend:

- research to determine minimum contact times at relevant concentrations for currently used sanitisers specifically to inactivate *L. monocytogenes* on the surface of whole melons, with consideration of the level of risk reduction both to consumers and the industry against practicality, economic, legal, and melon quality considerations

- validate commercial sanitisation processes using industry-relevant conditions of sanitiser concentrations, contact times and other variables (such as organic load) on inoculated whole melons

- *not* pursuing research into low penetration surface treatments such as UV and other light treatments, alone. However, in hurdle applications (using combinations of methods) there may be an application for these methods and, in general, research into the application of multiple hurdle/sanitisation technologies *is* recommended

- re-evaluating and initiating further research into methods that have demonstrated relatively high effectiveness against *L. monocytogenes* such as ozone, X-ray, octenidine dihydrochloride, hot water, superheated steam, and dry steam including determination of their costs versus benefits

- determining the efficacy of high penetration technologies, such as X-rays, to eliminate potential internal contamination of melons by *L. monocytogenes*

- future intervention studies should:

    - indicate the variety of melon used

- assess effectiveness against multiple pathogens (e.g. *L*. monocytogenes, *Salmonella,* and *Escherichia coli*) in parallel because they have been shown to have different resistance
- assess both the rind and the stem scar following inoculation and treatment because the efficacy of sanitisers has been shown to differ at these sites
- assess the effectiveness of treatments at multiple times after inoculation to determine the effect of biofilm formation on the effectiveness
- assess melon quality in parallel with pathogen inactivation tests to ensure that treatments are commercially viable
- perform re-inoculation and growth studies on whole melons after treatment to determine the capacity of *L. monocytogenes* to re-contaminate fruit from environmental sources
- use industry-relevant contact times, determine the effect of increasing levels of organic matter on efficacy, and apply inoculation and treatments to whole melons (rather than rockmelon portions)

Final comments

The rare outbreaks of listeriosis from rockmelons seem to be associated with a change in conditions in the field or the packhouse that introduces and/or concentrates the pathogen. If contaminated melons from the field then pass through or overwhelm the sanitising systems and no environmental monitoring or sufficient cleaning regimes are implemented, *L. monocytogenes* can colonise the packhouse unchecked and contaminate even ‘clean’ melons.

As this scoping study suggests, the efficacy of most of the current sanitising systems for whole rockmelons may be limited: even if those sanitising systems are optimised, *L. monocytogenes* may not be completely removed and may persist at low levels. Therefore, it is important for all procedures prior to sanitising to reduce the likelihood of the pathogen entering the sanitising system. Moreover, following sanitising, hygiene procedures must strive to prevent

recontamination of the fruit, and to reduce the potential for growth of the pathogen, and to prevent colonisation of the facilities by pathogens from the field or via other routes.

We have identified a range of potentially more effective sanitisers that warrant further research due to the potential they offer for improved risk reduction for both consumers and the industry. However, all will have limitations and, from our review of available literature and expert opinion, their overall effectiveness on rockmelon food safety will depend on the implementation of a vigilant and whole-of-supply-chain approach to food safety throughout the industry.

1 Introduction and rationale

The Australian melon industry is one of the larger Australian fruit industries, with a well-established production base across most Australian states and territories that ensures a year-round supply. Major production regions include Queensland (Bowen, Bundaberg and Chinchilla), New South Wales (Cowra and Riverina), the Northern Territory (Darwin and Katherine) and Western Australia (South Perth, Carnarvon and Kununurra). Victoria and South Australia produce relatively small volumes (<5% of national production). The southern production regions supply melons between January to July, while northern regions supply between July to December. In 2017/18, 215,519 tonnes of melons were grown valued at $124.2 million, of which rockmelons made up 25%.

Listeria monocytogenes is a pathogenic bacterium. It does not survive normal cooking, grows well at refrigeration temperatures, and is known to contaminate a variety of fresh produce, including melons. It has caused scores of major foodborne outbreaks, resulting in many deaths, miscarriages and severe illness, including a major outbreak from contamination of rockmelons in the USA in 2011 (147 cases, 33 deaths), and two rockmelon outbreaks in Australia (*see* also Section 6).

While there have previously been sporadic cases of listeriosis linked to rockmelons in Australia, a large outbreak occurred in Australia in early 2018 that resulted in 22 cases, 7 deaths, and 1 miscarriage (NSW DPI, 2018b). A smaller Australian outbreak that resulted in 9 cases and 2 deaths occurred in 2010. In the 2011 USA outbreak and 2018 Australian outbreak, while the affected rockmelons were in each case linked to a single grower, the consequences were felt by the entire industry and led to rockmelons being perceived as a high risk for food safety. As such, *L. monocytogenes* presents a hazard to the entire rockmelon industry, and identification and collation of the best growing, harvesting and post-harvest practices are required to assist both industry and consumers to reduce the risk from this pathogen. Further research is needed to identify or develop best practices to minimise the risks and promote public confidence in the safety and benefits of eating rockmelons.

Much is known about the ecology of *L. monocytogenes* and its behaviour on foods, the types of consumers at most risk of illness (very young, old, immunocompromised, pregnant), and the levels of *L. monocytogenes* required to cause illness. However, it is clear that the causes of the rockmelon listeriosis outbreaks must be better understood. From that, appropriate strategies to

prevent recurrences could be discerned, *e.g.,* more rigorous process controls, more reliable sanitisation of Australian rockmelons, and education of growers/processors about *L. monocytogenes* risk management options.

This project undertook a rigorous, quantitative and systematic analysis of current published literature and authoritative websites on the effectiveness of sanitisers against *L. monocytogenes* on rockmelons, on past listeriosis outbreaks associated with rockmelon and their causes, on strategies proposed and employed internationally to reduce the risk of listeriosis from rockmelons, and to relate that knowledge and experience to Australian conditions. This analysis is underpinned by knowledge of the ecology and pathogenesis of *L. monocytogenes*.

The project involved a team of experts with diverse experience in horticulture, food processing, and microbiological food safety, who consulted with industry stakeholders and were assisted by professional science communicators to ensure the delivery of robust but comprehensible findings and recommendations. The report also considers and discusses the limitations of reliance on testing for *L. monocytogenes* for product release as a means of food safety management and considers the relevance of a sustained environmental monitoring program for the growing area and processing environment.

2 Objectives

The Objectives of this review were developed by the expert team to ensure that the desired outcomes of Hort Innovation were achieved. The objectives were to identify, assess, synthesise, extend and clearly communicate the available knowledge related to risk assessment and risk management of *L. monocytogenes* on melons to the Australian melon industry. More specifically the objectives were:

1. to analyse the 2018 Australian listeriosis from melons outbreak and compare the causative factors in that incident to comparable international outbreaks from rockmelons by reference to published reports

2. from the available literature, and discussions with growers and other Australian melon industry stakeholders, to identify, analyse and summarise factors that can lead to contamination of rockmelons in the field, or elsewhere, with *L. monocytogenes*

3. to identify, analyse and summarise quantitatively all relevant reports on technologies for inactivation of *L. monocytogenes* on the surfaces of rockmelons (and other relevant fruits and other relevant pathogens)

4. to identify and scrutinise published (including 'grey literature') reports on the ability of *L. monocytogenes* to become internalised in rockmelons

5. to evaluate technologies, or other strategies, employed internationally for minimising the risk from *L. monocytogenes* in rockmelon

6. to address other key themes and issues identified during the project

7. to prepare industry-relevant reports and communications materials (in addition to the main report)

3 *Listeria monocytogenes* and rockmelons: background

While this literature review and analysis is principally focused on managing the risk of listeriosis from rockmelons through use of sanitisers or other decontamination technologies, it is also relevant to place the risk of human listeriosis from consumption of rockmelon in context so that the most effective holistic risk management strategies can be identified and implemented.

At the outset, however, it should be noted that while rockmelons have been involved in numerous food-borne disease outbreaks internationally, most usually from *Salmonella* spp. or norovirus, *there have only ever been three reported outbreaks of listeriosis from rockmelons*: two in Australia (2010, 2018) and one in the United States of America (2011). However, while listeriosis is a rare disease it is frequently fatal. The 2010 Australian outbreak caused nine invasive cases and two deaths (FSANZ, 2011). The 2018 Australian outbreak caused 22 cases, including 7 deaths and 1 miscarriage. The 2011 USA outbreak caused 147 cases of invasive listeriosis, including 1 miscarriage and 33 deaths. It is also notable, though currently unexplained, that many more foodborne outbreaks (whether from *L. monocytogenes* or other pathogens) from melons are reported in the USA than in the European Union (Callejón et al., 2015). Australia also experienced a large outbreak (144 cases) (C. Shadbolt, pers. comm., 2020) of salmonellosis in 2016 from rockmelons grown in the Northern Territory (NSW DPI, 2018a) and another in 2006 that involved 100 cases and one death (FSANZ, 2011).

3.1 Eco-physiology of *L. monocytogenes*: relevance for rockmelons

There are numerous reviews of the eco-physiology of *L. monocytogenes* and its relevance to foods (Buchanan et al., 2017; FSANZ, 2013; ICMSF, 1996; Ross et al., 2000; Sauders and Wiedmann, 2007; Vivant et al., 2013; Zhu et al., 2017; Marik et al., 2019). *L. monocytogenes* is a Gram-positive bacterium that grows at refrigeration temperatures, with reports of growth at temperatures as low as -1°C. Its upper temperature limit for growth is ~45°C. It is also salt tolerant, and can grow at salt levels up to 11 – 12% (aqueous phase, corresponding to a water activity of 0.92 – 0.93) (Shabala et al., 2008), and also endures desiccation better than many other bacteria. It is also able to grow at pH > 4.2 – 4.3 (Shabala et al., 2008) and up to pH 9.5 (FSANZ, 2013; ICMSF, 1996). But it is nutritionally 'fastidious' (Premaratne et al., 1991; Sauer et al., 2019) preferring a range of preformed organic nutrients, and particularly free amino acids and some vitamins, to enable it to grow most abundantly. *L. monocytogenes* is effectively a saprophyte and is frequently associated

with decaying organic vegetation, including silage, in which other organisms contribute to the breakdown of more complex organic compounds into simple sugars and free amino acids that *L. monocytogenes* is more readily able to utilise. Numerous studies have shown that it is readily detected in many natural environments and can also be found in the faeces of birds and animals, including humans, most of whom appear to be transient carriers. It can also be found in soils, albeit at relatively low levels, provided that the soil is moist, and can infect soil-borne protozoans that may facilitate its growth and dispersion (Vivant et al., 2013). For this reason, *L. monocytogenes* is considered ubiquitous in the environment and, particularly, in moist environments. It is a particular problem in the ready-to-eat food industry because food processing plants are often wet, contain decaying food particles in cracks and crevices in buildings and equipment, and are cold: all of these conditions match the natural environmental niche of *L. monocytogenes.* These food-processing plant niches also provide sources of contamination and, even though *L. monocytogenes* is readily killed by heat (*see below*), such sources of contamination can re-contaminate ready-to-eat foods even after they have been pasteurised or cooked (e.g., cheeses, smallgoods). Despite that it is generally considered that the natural environmental niche of *L. monocytogenes* is *not* in mammalian hosts, it is able to cause severe human illness, particularly in people with weakened immune systems and exhibits two modes of existence, changing its physiology when in the environment or a mammalian host (Toledo-Arana et al., 2009).

Because rockmelons contain simple sugars and can be damaged in the field, damaged rockmelons in the field could also become contaminated with *L. monocytogenes* and transfer contamination to the pack-house if damaged melons are not removed before processing. It is also possible that *L. monocytogenes* can become internalised in rockmelon *via* the stem (*see* Section 9.3).

L. monocytogenes is readily inactivated by heat and, as such, is mainly of concern to foods that are sold as 'ready-to-eat'. i.e., not requiring further cooking before eating. D-values (the time required at a given temperature for a one-log (90%) reduction) for *L. monocytogenes* have been presented by various authors and organisations (Bunning et al., 1988; Coote et al., 1991; FDA, 2019b; NZMPI, 2016; van Asselt and Zwietering, 2006). Whilst there is variation in the reported results due to strain differences, and the methods used to measure inactivation, at 65°C the time required for a 90% reduction in *L. monocytogenes* levels in a variety of foods is typically 1 – 3 minutes, and at 74°C is typically 1 – 6 seconds. At 70°C, the time required is 5 – 30 seconds. The z-value (temperature change required for a ten-fold change in inactivation rate) for *L. monocytogenes* is

variously reported to be in the range 6 – 8°C (Bunning et al., 1988; FDA, 2019b; van Asselt and Zwietering, 2006). Coote et al. (1991) noted that "the heat resistance of *L. monocytogenes* is comparable with that of many other non-sporing mesophilic bacteria", an observation supported by the results of van Asselt and Zwietering (2006).

3.2 Dose-response relationships for *L. monocytogenes*

To understand how best to manage the risk of listeriosis from any kind of food, including rockmelon, it is necessary to understand the relationship between the dose of *L. monocytogenes* ingested and the probability of a consumer falling ill. This information is termed the 'dose-response relationship'. The dose-response relationship is often summarised as a mathematical equation, or 'model'[1]. There are various mathematical models used to describe dose-response relationships and based on different hypotheses concerning the processes of exposure to a pathogen and the subsequent processes of infection and resistance to infection in the human host. Those various models are considered in detail in FAO/WHO (2003) and, in the specific case of *L. monocytogenes*, in FAO/WHO (2004).

FAO/WHO (2003) considered that the most plausible and scientifically-defensible model to describe the relationship between the dose of a pathogen that is ingested (i.e., the total number of viable cells eaten by a person in a meal) and the probability of that person becoming ill is the 'exponential model', which has the form:

$$P_{illness} = e^{(-r \times \text{dose ingested})}$$

where*:*

'$P_{illness}$' is the probability of illness,
'dose ingested' is the number of cells of the pathogen consumed in a meal (a function of meal size and level of contamination)
'*r*' is a parameter that is specific to the pathogen of interest (i.e., whether *L. monocytogenes*, *Salmonella*, norovirus, etc.) and that effectively describes the probability that a single cell of the pathogen could cause illness.

1 The term 'model' here is for a mathematical equation that describes our understanding of how a system works and how it responds to influences or changes. The model generally quantifies the effects of variables that influence the system, or the parameter(s) in the system in which we are most interested. The factors that influence the outcome (e.g., pathogen virulence) are termed parameters and their interactions are described by mathematical expressions (addition, multiplication, exponentials, etc). The same structure of mathematical equation can describe our general understanding of a process (e.g., dose response relationship) but is made specifically for a particular example by determining the co-efficients and parameters that are specific to the situation, e.g. for the pathogen and population group of interest. Thus, a single dose-response model structure can apply to all infectious agents but is made specifically for a particular pathogen by determining the values of the parameters and co-efficients that are characteristic to the pathogen of interest.

Therefore, if the '*r*' value is 10^{-6} (or, 0.000001), there is approximately a one-in-a-million chance that a single cell could cause infection. Alternatively, there would be about a 50% chance of illness if a person consumed 500,000 cells (e.g., 100g of food containing 5,000 CFU/g).

From the above, the dose that would be required to cause illness in 50% of consumers, i.e., the average consumer, can be calculated more specifically by determining the dose ingested that results in $P_{illness} = 0.5$. That value, specific to each pathogen, is called the ID_{50} (the infectious dose that would cause illness in 50% of consumers). The ID_{50} value is useful for comparing the virulence of pathogens: a lower ID_{50} indicates a more virulent pathogen. At doses below the ID_{50}, the exponential model predicts an almost direct proportionality between the dose ingested and the probability of illness. At doses above the ID_{50} the relationship between higher doses and probability of illness rapidly reaches an asymptote, or 'plateau', as shown in Figure 1.

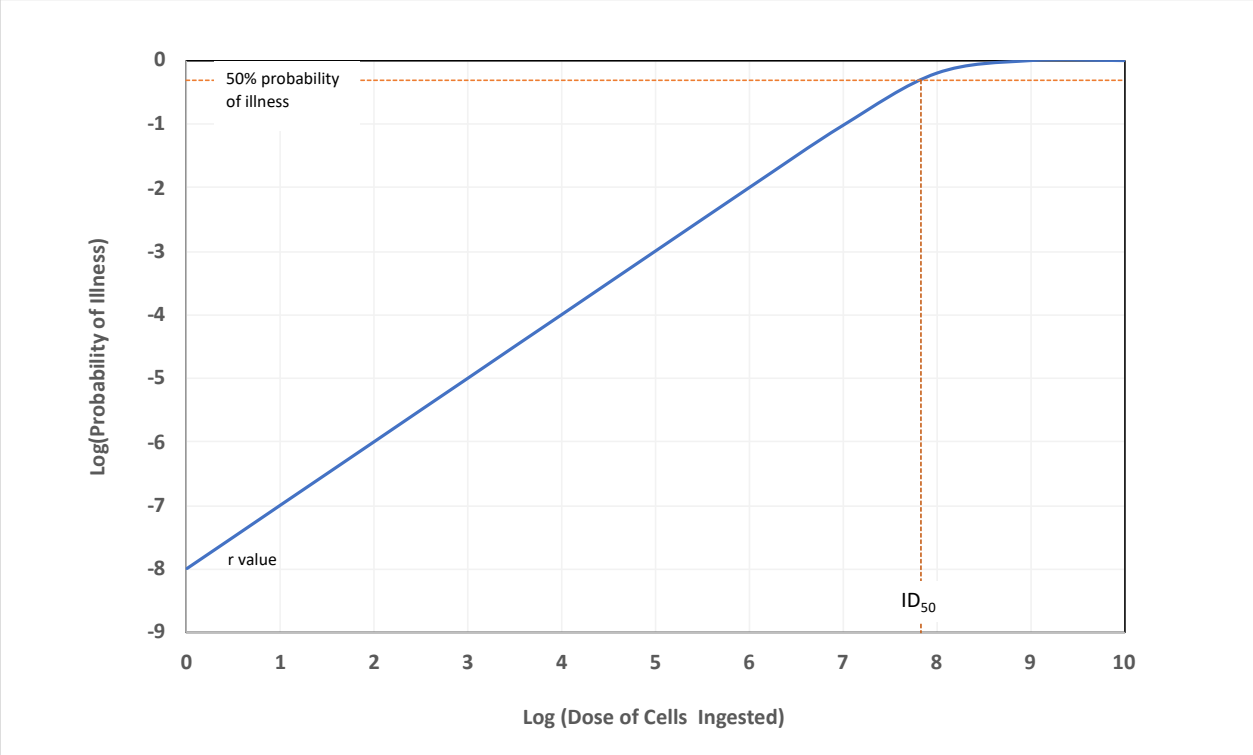

Figure 1. A dose-response relationship for the probability of illness upon consumption of different doses of a pathogenic bacterium. Also shown is the relationship between the ID_{50} and 50% probability of illness and the '*r*' value for the dose response relationship. The dose and probability of illness data are presented on logarithmic scales for clarity and ease of interpretation.

In the case of listeriosis it is not possible to conduct feeding trials with humans to determine the dose-response relationship because the outcome of infection is often fatal for the consumers of greatest interest (i.e., the 'YOPI' group, which are the **Y**oung, **O**ld, **P**regnant or **I**mmunocompromised - in most developed countries the YOPI groups constitute about 15 to 20% of the total population - discussed more fully below). Accordingly, because listeriosis outbreaks are of such great concern for the ready-to-eat food industries (e.g., dairy, smallgoods, lightly preserved seafoods and, latterly, fresh produce industries) due to the potential severity of the consequences for consumers, there have been numerous attempts to develop dose-response relationships for foodborne *L. monocytogenes* that have been based on other data such as:

- outbreak data, often based on summaries of older data (CFSAN/FSIS, 2003; FAO/WHO, 2004; Pouillot et al., 2014),
- analysis of more recent specific outbreaks (Pouillot et al., 2016)
- attempts to match the incidence of listeriosis in a community to the prevalence and the predicted contamination levels of *L. monocytogenes* in that community's food supply (Buchanan et al., 1997; FAO/WHO, 2004) or, latterly,
- using *relevant* pregnant animal models (Roulo et al., 2014; Smith et al., 2008; Smith et al., 2003; Williams et al., 2009; Williams et al., 2007)

From published analyses of outbreak data (CFSAN/FSIS, 2003; FAO/WHO, 2004; Pouillot et al., 2014) there is a very wide spread of ID_{50} estimates (*see* e.g., (FAO/WHO, 2004), Fig. 2.7, p. 49 of that report). This probably relates to variability in strain virulence and variability in host susceptibility: those estimates range from approximately 10^6 cells to $>10^{11}$ cells, depending on the outbreak data considered.

As mentioned above, due to its mode of pathogenesis, listeriosis most often affects people that are immunocompromised or immunodeficient (e.g., by virtue of pregnancy, the elderly, or very young babies) or immunocompromised due to disease (e.g., HIV/AIDS, leukaemia, alcoholism, cirrhosis, or medications to prevent rejection of organ transplants). Based on epidemiological data from cases of listeriosis in France from 2001 - 2008, Goulet et al. (2012) undertook an analysis of the relative susceptibility of consumers with known conditions that predispose them to listeriosis compared to the rest of the population that do not have predisposing conditions. Their study was

based on 1959 cases of listeriosis in France over the period 2001 – 2008 and considered 37 predisposing factors. They found that the relative susceptibility for pregnant women is 116 times greater than for people less than 65 years old with no known predisposing conditions. For people 65 -74 years old the relative risk is eight times greater; for people over 74 years old the relative risk is 20 times greater; for people with a range of cancers the average relative risk is 78 times greater, but for people with liver cancer or chronic lymphocytic leukaemia (CLL) the relative risk is 748 or 1139 times greater, respectively. People with Type 2 diabetes are 3 times more susceptible to listeriosis than the average, healthy, adult. To put some of these relative susceptibilities into context, in the period 2001 to 2008 the French population increased from 61 to 64 million people. During that time 774,000 women were pregnant (noting that this proportion seems relatively low for the growth rate of the French population during that time), 3.5 million people over 74 years old, 2.25 million people living with some form of cancer, 9000 people with liver cancer, and 20,000 people with CLL, while there were 2.5 million people with Type 2 diabetes. In Australia in 2019, 14% of the population were older than 65 years and another 2% older than 85 years (ABS, 2019) and about 1% of people were pregnant at any given time[2] .

In general, from those data, pregnant women (and their unborn babies) represent a group that has a 'moderate' to 'high' level of susceptibility to listeriosis as well as representing a moderate sized sub-population of potentially susceptible people. As such pregnant women are generally representative of the relative risk of listeriosis among susceptible populations and we will use them in the discussion below to exemplify the listeria dose-response relationship and risk to consumers from listeria-contaminated foods.

Following the approach of Buchanan et al. (1997), FAO/WHO (2004) used a complex stochastic procedure to infer an exponential dose-response model for *L. monocytogenes* from the expected levels of *L. monocytogenes* for 20 categories of ready-to-eat foods in the USA and consumption frequencies of those foods. Those expected levels were based on survey data for *L. monocytogenes* in foods in the USA, as reported in (CFSAN/FSIS, 2003). From that modelling they inferred that the 'r' value is 2.37×10^{-14}, corresponding to an ID_{50} of 2.9×10^{13} cells for a healthy adult individual. For susceptible individuals (e.g., pregnant women) the probability of infection from a single *L. monocytogenes* cell was estimated as 1.06×10^{-12} corresponding to an ID_{50} of 6.5 x

2 In Australia about 0.92% of the population is pregnant at any given time (e.g., 315,147 births in Australia in 2018) in a population of 25 million (ABS, 2018), each pregnancy lasts ~9 months, and about one in 80 pregnancies involves twins.

10^{11} cells, or approximately 45-fold greater risk. Earlier studies used rats and mice to try to establish the risk of infection (though not necessarily invasive listeriosis) for various oral doses of *L. monocytogenes* (*for review see* FAO/WHO (2004)) and found great variation in doses required to establish infections. However, rats and mice are now known not to be representative of the processes of human infection by *L. monocytogenes* because they lack the E-cadherin protein on their epithelial cells. E-cadherin is specifically bound by Internalin proteins on the outside of *L. monocytogenes* and that binding is a key initial step in the infection process (Bonazzi et al., 2009).

Animal models, using pregnant mammals (Roulo et al., 2014; Smith et al., 2008; Smith et al., 2003; Williams et al., 2009; Williams et al., 2007) that do have the E-cadherin protein on their epithelial cells (as do humans), have tended to produce more consistent results, and suggest that LD_{50}[3] for foetuses are in the range 10^6 to 10^8 cells of *L. monocytogenes* (using known virulent strains) administered orally to the mother.

The data and analyses presented in Pouillot et al. (2016), based on a well-characterised outbreak in the USA from ice-cream, allow estimates of the ID_{50} of the 'average' population to be made. Based on their estimates, the ID_{50} for the 'average' consumer ranges from 2.7 x 10^8 cells (from their low consumption estimate) to 2.4 x 10^9 cells (from their high consumption estimate), or 3 x 10^7 to 3 x 10^8 for pregnant consumers. As expected from the above discussion, the ID_{50} estimates for pregnant consumers are lower by a factor of 10, which is lower than, but relatively consistent with, the analysis by Goulet et al. (2012).

While the various analyses have used different measures of disease incidence (i.e., ID_{50} or LD_{50}), it is apparent that the more recent analyses with appropriate experimental models suggest that the ID_{50} or LD_{50} for *L. monocytogenes* for pregnant mammals and their foetuses, via oral ingestion, are in the range 10^6 to 10^8 cells of *L. monocytogenes* for susceptible populations, though probably much higher (~100-fold) for people in good health who have no identifiable risk factors for listeriosis. To further reinforce this point, all the most recent estimates of ID_{50}/LD_{50} for *L. monocytogenes* are much lower than the estimates presented in CFSAN/FSIS (2003) or FAO/WHO (2004). Also, for comparison, other studies have suggested that the ID_{50} for salmonellosis (based

3 LD_{50} stands for 'lethal dose that will cause death in 50% of consumers. It is analogous to the ID_{50} but uses death as the endpoint, rather than the simple establishment of infection.

on outbreak data) is ~10,000 cells (FAO/WHO, 2002) and for norovirus infections is ~20 virus particles (Teunis et al., 2008).

3.3 Risk management considerations

Despite the relative ubiquity of *L. monocytogenes* in the environment and on foods (CFSAN/FSIS, 2003) listeriosis is a rare infection. In Australia, there are typically 60 – 70 cases of listeriosis (almost always food-borne) per year (NNDSS, 2020) and it is considered that underreporting of invasive listeriosis is relatively low (~50%) because of the disease severity and that most victims will need to seek medical attention (Scallan et al., 2011). Invasive listeriosis is a severe infection and results in fatality in 20 – 30% of cases (FAO/WHO, 2004). Thus, as explained earlier, minimising the risk of consumer exposure to dangerous levels of *L. monocytogenes* in ready-to-eat foods is critical to both public health and the longevity and reputation of relevant food industries, including the Australian rockmelon industry.

In simple terms, and as embodied in the "ICMSF equation" that embraces the idea of Food Safety Objectives (FSO) (van Schothorst et al., 2009), to manage the risk, the combination of the prevalence and levels of pathogens in foods at the time of consumption should be low enough to achieve an Appropriate Level of Protection (ALOP) to consumers, i.e., that the risk of illness upon consumption of a food is low. The ALOP can be achieved by keeping the frequency of contamination low, or by keeping the *level* of the contaminant, if present, low. This can mean minimising contamination, reducing contamination by treatments such as sanitisers, and/or minimising growth on the food. As discussed above, the ID_{50} of *L. monocytogenes* is relatively high, but growth is possible both on the rind (if the relative humidity of the storage environment is high or if the fruit remains wet – *see above and also* Section 9.2) and, more certainly, on the flesh once the fruit is cut, even at refrigeration temperatures: growth to dangerous levels can occur in a day if rockmelon is cut and then left at ambient temperature (*see* Section 9.2). Thus, to ensure the safety of rockmelon for consumers and the viability of the industry, contamination frequencies and levels need to be minimised as does the potential for growth of *L. monocytogenes* on the product. Educating consumers about appropriate handling of melons after cutting them (i.e., to then store them under refrigeration) will also be a useful component of the overall risk management, e.g., in the 2018 listeriosis from rockmelons outbreak in Australia, it was noted that all cases occurred in consumer's homes, not in "care-facilities" (NSW DPI, 2018b).

The FSO concept is predicated on a whole-of-supply chain approach to food safety management. The potential for growth of *L. monocytogenes* throughout the supply chain can be limited by refrigeration and water/relative humidity control on the fruit, and also relies on appropriate consumer knowledge and practices. Nonetheless, the producer-processor has a key role to play in minimising initial contamination, especially for ready-to-eat foods.

While disinfection of melons (*see* Section 8) can reduce the prevalence and levels of *L. monocytogenes,* methods and technologies to prevent contamination in the packhouse, and potential transfer of that contamination to fruit, appear to be equally important. As noted above, and detailed in Section 6, the available evidence from outbreaks suggests that contamination of rockmelons within the field is unlikely as a cause of outbreaks except that it might lead to contamination of the processing plant/packhouse itself and that *that* might lead to large-scale outbreaks if contamination and colonisation of the packhouse goes undetected for a prolonged period of time.

Accordingly, while minimisation of *L. monocytogenes* on rockmelons by disinfection will be important in minimising the risk of plant colonisation, environmental monitoring programs (to detect *L. monocytogenes* colonisation in packhouses) are likely to be an important component of risk management for the Australian rockmelon industry. Strategies for environmental monitoring are considered in Section 10 of this report.

As will be discussed in Section 6 of this report, outbreaks of listeriosis from whole rockmelons are rare, with only three reports globally in the last 40 years found by our Literature Review (*see* Section 4). Notably, *Salmonella* outbreaks from rockmelons are much more common internationally, and particularly in USA/Canada, but have been reported only twice in Australia (Munnoch et al., 2009; NSW DPI, 2018a). Given that rockmelons have been produced and consumed in Australia for decades without having caused frequent outbreaks of listeriosis, it may seem relevant to ask why the Australian rockmelon industry should be concerned. The answer is that the consequences of the 2018 listeriosis outbreak for the entire Australian industry, were dire (*see* Section 6.2), as were the effects of the 2016 *Salmonella* Hvittingfoss outbreak (NSW DPI, 2018a). While the frequency of such outbreaks is rare, the consequences for the industry are such that the industry has to be proactive in preventing them from occurring.

The colonisation of packhouses by *L. monocytogenes* represents a significant threat to the industry. Using sanitisers to remove contamination on rockmelons will greatly reduce the chances of contamination, as well as reducing potential contamination of fruit with enteric pathogens such as *Salmonella* and pathogenic *Escherichia coli* (i.e., that are more likely to arise from environmental contamination of fruit by animals and birds). However, contamination of fruit in the field with human pathogens can be a dynamic process: available evidence suggests that weather events (*e.g*., dust storms, heavy rainfall) can contribute to *greater* than usual contamination of rockmelons (NSW DPI, 2018b), whether with enteric pathogens or *L. monocytogenes.* Importantly, such contamination might overwhelm normal hygiene/disinfection processes within the packhouse. In such cases, it may be prudent to apply more stringent sanitisation methods, *e.g.,* longer contact times or higher sanitiser concentrations if unusual events occur that could cause higher than normal contamination levels. This is also alluded to in the melon industry 'best practice' documents (*see* Section 7).

As is discussed in Section 8, most currently available *practical* sanitisation methods will achieve 1 – 3 $\log_{10}$CFU inactivation/removal of *L. monocytogenes*, which may not provide the level of consumer safety needed. While further research on improved technologies with greater efficacy is needed, a solution in the interim can include the application of 'Hurdle Technologies', to use multiple treatments in combination, or to implement better supply chain management (e.g., refrigeration, humidity control to minimise growth) or to apply multiple disinfection technologies sequentially.

Importantly, there are other changes that will probably affect the likelihood of further listeriosis outbreaks both from occurring and from being recognised. These include the increasing proportion of susceptible (i.e., immunocompromised) people in the Australian community and particularly the elderly: in other words, even low frequencies of contamination could lead to many susceptible people being exposed and becoming ill. Secondly, the increasing scale of production/processing through single pack-houses means that, if there is a contamination problem in that packhouse, more people will be exposed which could lead to an overt outbreak. Thirdly, there have been powerful advances in our ability to specifically detect common-source outbreaks, mainly through the development and adoption of whole genome sequencing (WGS)

technologies in combination with active disease surveillance networks, e.g., OzFoodNet[4]. The power of WGS for identification of common-source food-borne disease outbreaks, including the unambiguous identification, and resolution, of the sources of the 2011 USA outbreak and 2018 Australian listeriosis outbreaks has been noted by many reports (NSW DPI, 2018b; McCollum et al., 2013; Garner and Kathariou, 2016; Desai et al., 2019; Buchanan et al., 2017). Similarly, WGS provided unambiguous identification of the source of the 2016 *Salmonella* Hvittingfoss outbreak in Australia (NSW DPI, 2018a). WGS will likely be able to detect smaller, more diffuse, outbreaks and those that are spread out over longer periods. This has recently been demonstrated in a small outbreak of listeriosis related to cold smoked salmon in Australia (3 cases, across three Australian states), but that caused two deaths (ABC, 2020). In that case, WGS provided the evidence for a common source outbreak and initiated the investigation that eventually found the source, a single food producer in another Australian State.

4 OzFoodNet is an Australian national food-borne disease surveillance program that aims to identify foodborne outbreaks rapidly and take steps to minimise their effect on public health. For more information see: https://www1.health.gov.au/internet/main/publishing.nsf/Content/cdna-ozfoodnet.htm

4 Approach for the review

The review of the literature followed the methodology of Colquhoun et al. (2014). A flowchart of the review process is presented in Figure 2.

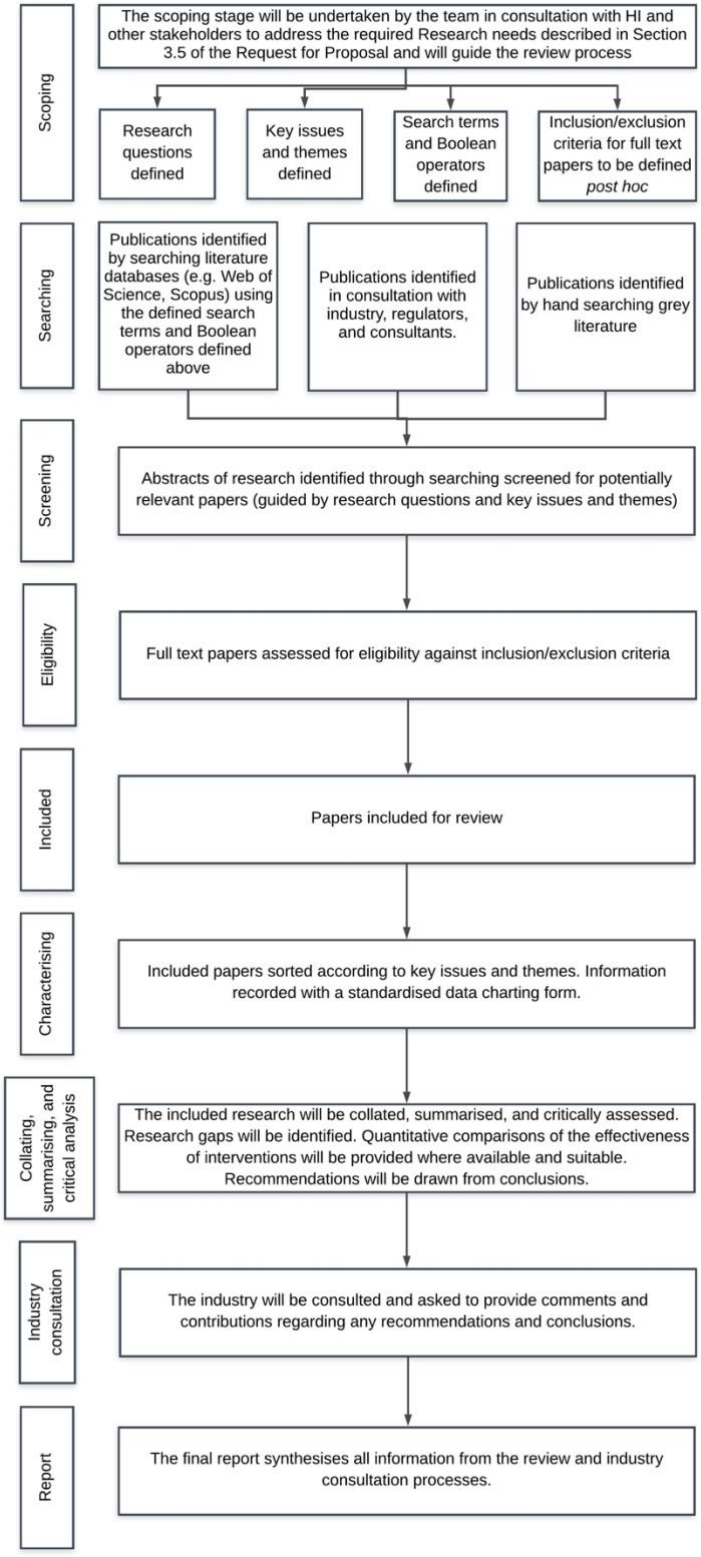

Figure 2. Flow chart detailing the steps of the review process.

4.1 Research questions, scope, definitions, and key themes

The team members met 'virtually' to determine the scope, research questions and key themes of the review process. The following research questions and scope were agreed upon to achieve the objectives outlined in Section 2:

The research questions and scope for the review of previous outbreaks were defined as follows:

1. Identify the most recent (2010 – present) confirmed or potential outbreaks that have occurred from contamination of rockmelon/melons by *L. monocytogenes* and *Salmonella* in Australia, North America, or Europe.

2. Articulate the key findings regarding the route of *L. monocytogenes* contamination of rockmelons, and whether there are similarities or differences across the rockmelon outbreaks identified.

3. What were the key outcomes or recommendations that arose following the investigations of the identified outbreaks?

The research questions and scope for the review of best practice recommendations, primary production interventions, growth rates, prevalence, and internalisation were defined as follows:

1. What 'best practice recommendations', have previously been provided to the rockmelon industry, both in Australia and internationally, that aim to reduce the prevalence of *L. monocytogenes* (from primary production to when melons leave the farm gate)?

2. What research exists describing the effectiveness of different interventions to minimise/control *L. monocytogenes* in whole melons at all stages from primary production to when melons leave the farm gate? What data gaps exist, what emerging technologies may be applicable, and where is further research required? (Where papers provide information for other pathogens, in addition to *L. monocytogenes*, they will also be reported.)

3. What research exists regarding growth rates, prevalence, or internalisation of *L. monocytogenes* in/on rockmelons from primary production to consumption. What data gaps exist and where is further research required?

Processes for fresh-cut melons and interventions after melons leave the farm gate were beyond the scope of this study.

The following definitions were defined and agreed upon:

'Melons': Cantaloupe, rockmelon, muskmelon, watermelon, honeydew melon.

'Best practice recommendations': Suggested methods or techniques for pre- and post-harvest melon production that aim to reduce microbial contamination and that have been provided by government, regulatory, or other authoritative organizations.

'Intervention': additional control measures undertaken or applied during pre- or post-harvest of melons to reduce *L. monocytogenes* on the surface of whole melons.

The key themes to characterise relevant publications were identified as:

- Australian and international outbreaks associated with melons and *L. monocytogenes* from 2010-to-present
- Best practice recommendations proposed to the melon industry in Australia and internationally
- Pre-harvest interventions to reduce *L. monocytogenes* contamination of whole melons
- Existing, already adopted, post-harvest interventions (e.g., exterior melon sanitising) to reduce *L. monocytogenes* contamination of whole melons
- 'Alternative' post-harvest interventions – other interventions to reduce *L. monocytogenes* contamination of whole melons
- Growth rates, prevalence, or concentration of *L. monocytogenes* in or on rockmelons from primary production to consumption
- Internalization of *L. monocytogenes* in melons

Before undertaking the review, to provide context and to understand the growing and processing conditions in the Australian rockmelon industry, we visited farms and packhouses in eastern Australia (NSW and Far North Queensland), and Western Australia (Carnarvon and Perth region).

We interviewed growers/producers and other stakeholders to understand their needs and capability to implement anti-listerial programs, and for their experience and learnings on practices that affect food safety outcomes for rockmelons. This allowed us to include valuable industry knowledge in the initial scoping stage of identifying key issues and themes that guided the review process, as well as giving the team members (without those perspectives) the necessary background/understanding to be better able to assess the relevance of the literature information to Australian industry conditions. We attempted invite all farms that were unable to be visited to provide information via email, post, or phone. Other industry stakeholders were also invited to provide information or highlight areas of interest that might be within the scope of the review.

4.2 Search terms, search strategy, and review of relevant literature

The search strategy was designed to undertake an exhaustive search that would identify all relevant literature regarding the research questions described in Section 4.1. The search terms with the Boolean operators that were developed are presented in Appendix 1. Searches with those terms were undertaken in two electronic bibliographic databases; Web of Science and Scopus. Grey literature, including industry and regulatory websites, books, and Google Scholar were also searched. Experts and industry stakeholders were also consulted and asked to identify relevant literature. The citations from all searches were collated in a single folder, imported into reference manager software, and then de-duplicated. The reference lists of articles identified as relevant were searched for additional relevant publications.

The abstracts of all papers identified during the search stage were screened to identify the relevant papers and the screening process (described in Appendix 2) was developed with reference to the research questions, lessons learned from industry visits, stakeholder discussions, and the key issues and themes identified. Following abstract screening, the eligibility of papers to be included in the review was assessed based on post hoc inclusion/exclusion criteria (described in Appendix 2) applied after reading the full text versions. Inclusion/exclusion criteria were initially developed during the first team meeting, however, were adapted during the screening/eligibility process as we became more familiar with the subject matter.

The papers selected for inclusion in the review were then sorted by characterising according to the identified key themes (described in Section 4.1). All selected papers were then critically assessed, summary information recorded, and quantitative data for post-harvest interventions recorded in a standardised format to enable comparison of efficacy. Specifically:

- Quantitative data describing reductions in pathogen loads following the application of interventions were extracted from tables in publications or derived from graphs using the online tool WebPlotDigitiser (available at: https://automeris.io/WebPlotDigitizer/).
- Where a publication provided results for other pathogens, in addition to *L. monocytogenes*, they were also be reported.
- Where log_{10}CFU reductions were not reported in a paper, the mean surviving numbers of pathogens after applications of interventions were compared with the mean numbers of pathogens on inoculated untreated control melons with all units converted to either Log_{10} CFU/(g or cm^2).
- Where the '>' symbol appears in front of a log_{10}CFU reduction value in the tables, this indicates a result where the pathogen was reduced to below the limit of detection (LOD), i.e., that it is possible for the actual inactivation to be a larger than what is reported (This limitation is further discussed in Section 11).

All information included in the review was critically assessed by the panel of experts who provided further feedback and advice.

As we aimed to represent the full breadth of relevant research, there was no systematic quality assessment step to exclude 'unreliable' papers. However, the limitations of particular papers have been commented on in this literature review.

The conclusions and recommendations of the study were discussed and developed by the expert panel. A draft of the final report was released to industry stakeholders for comment before the research team produced the finalised document.

5 Overview of the results of the literature search underpinning this report

The process and results of the review are summarised in Figure 3. Searches were undertaken from October 1st 2019 to December 30th 2019. A total of 1181 papers were initially identified and, following deduplication, relevance screening, and characterization, 87 papers were included in the review. The publication dates of these papers ranged from 2002 to 2019 as detailed in Figure 4. Most papers described post-harvest interventions to reduce *Listeria* on the surface of melons.

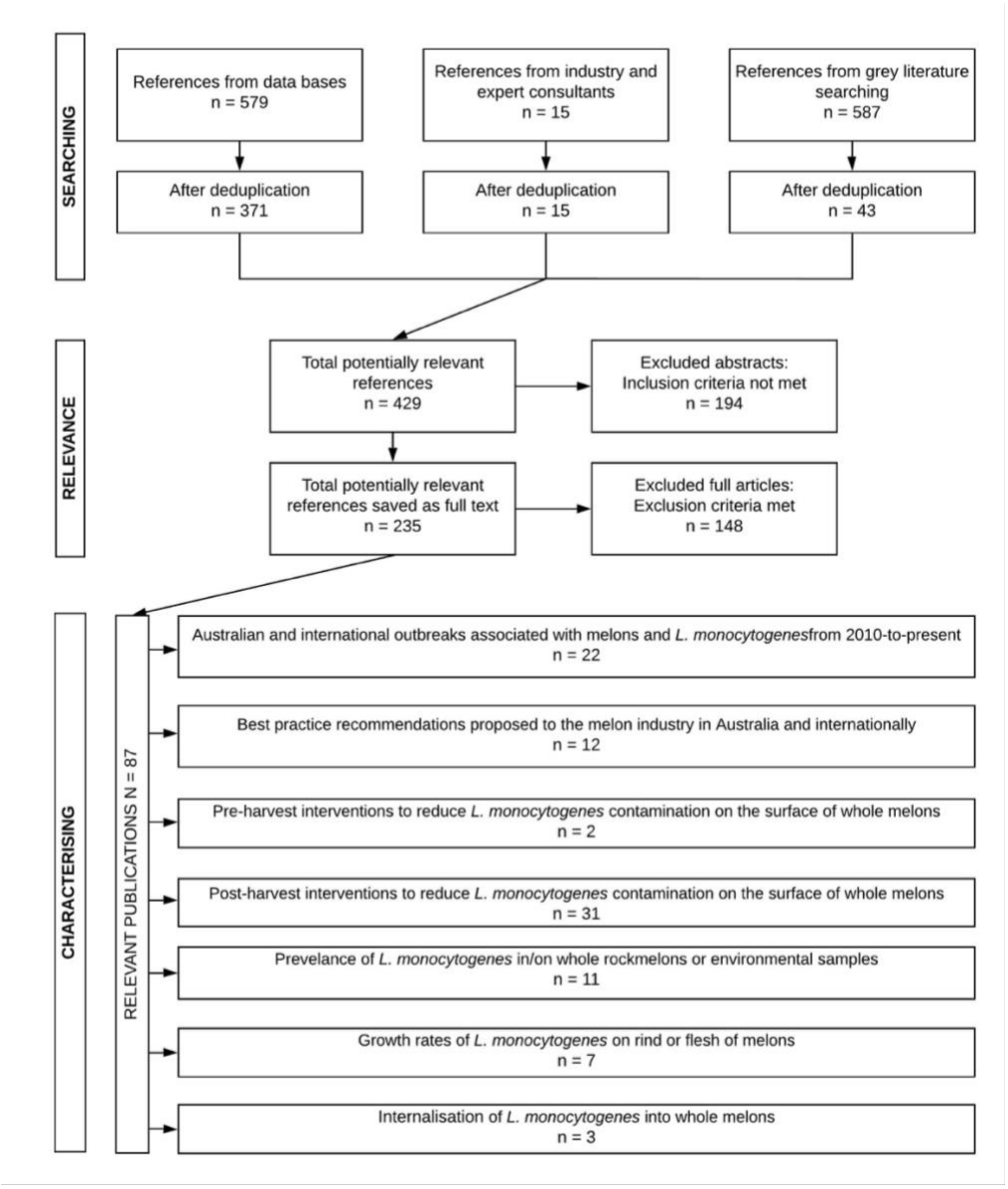

Figure 3. A summary of the searching, screening, and characterisation process and the number of studies identified at each step. (n.b., the number of "relevant" publications (87) is less than the total of the papers by theme because some papers include information on multiple themes).

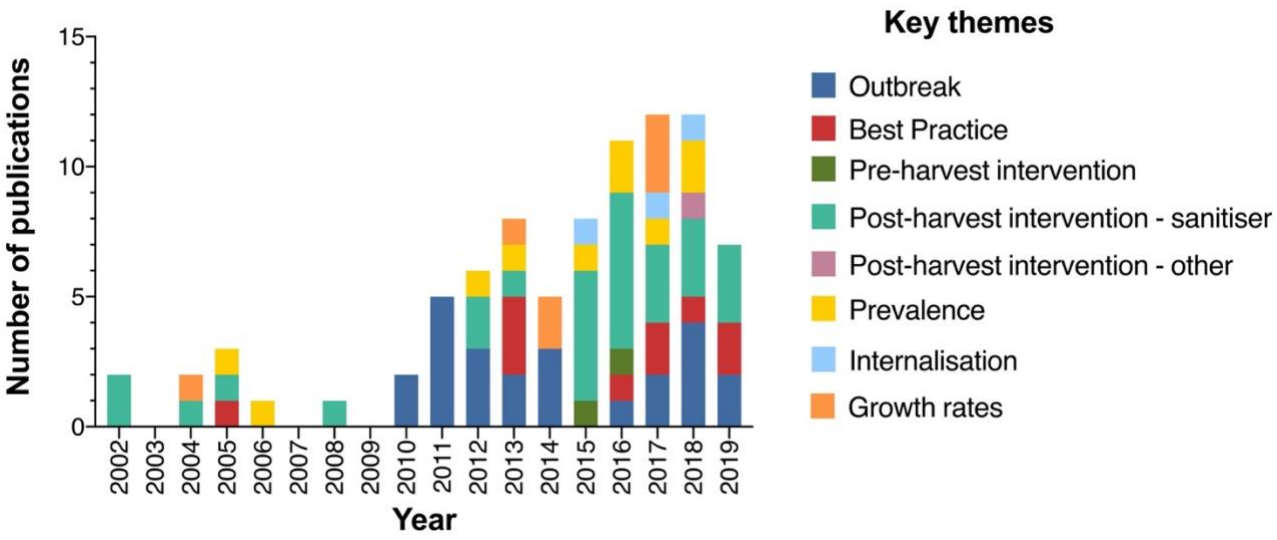

Figure 4. The number of studies identified in the review by year and key theme. It should be noted that only international outbreaks following 2010 were included in the review.

We have presented the detailed summaries and interpretation of the available authoritative research in this report under four different Section headings:

- Section 6: Review of previous outbreaks of *L. monocytogenes* or *Salmonella* associated with melons
- Section 7: Review of previous 'Best Practice Recommendations' provided to the melon industry
- Section 8: Review of pre-harvest and post-harvest interventions for reducing the risk of *L. monocytogenes* on whole melons
- Section 9: Review of prevalence, growth rates, and internalisation of *L. monocytogenes* in/on whole rockmelons from primary production to consumption

6 Review of previous outbreaks of *L. monocytogenes* or *Salmonella* associated with melons

The objective of this review is to identify the most recent (2010 – present) confirmed or potential outbreaks that have occurred from contamination of rockmelon/melons by *L. monocytogenes* and *Salmonella* in Australia, North America, or Europe. We critically assessed and summarised the key findings regarding the potential routes of *L. monocytogenes* contamination of rockmelons.

The literature review identified twenty-two authoritative reports concerning nine outbreaks related to melons and *L. monocytogenes* or *Salmonella* from 2010 to 2020. Those outbreaks and the probable causes reported by authoritative agencies are summarised in Table 1.

A further fifteen studies and reviews that have summarised findings regarding outbreaks involving *L. monocytogenes* and melons were also considered relevant and are included in the discussion of these outbreaks that follows. Of those outbreaks, two occurred in Australia (in 2010 and 2018). The 2010 outbreak in Australia resulted in nine cases and two deaths (FSANZ, 2011). The 2018 outbreak in Australia involved 22 cases and caused seven deaths and one miscarriage (NSW DPI, 2018b). The other reported listeriosis outbreak involving rockmelons occurred in the USA in 2011 and led to 147 cases and 33 deaths (McCollum et al., 2013).

Among foodborne disease outbreaks internationally that are linked to fresh produce (*see* Zhu et al., 2017; Walsh et al., 2014; NSW Food Authority, 2013; McCollum et al., 2013; Gagliardi et al., 2003; FSANZ, 2011; FAO/WHO, 2011; EFSA/ECDC, 2018; Desai et al., 2019; Danyluk et al., 2014b; Callejón et al., 2015; Buchanan et al., 2017; Bowen et al., 2006) melons are frequently implicated, although listeriosis outbreaks are rare (*see* e.g., Table 1). For example, when considering all fresh-fruit related outbreaks in USA, norovirus is the most common cause of outbreaks (Callejón et al., 2015). Walsh et al. (2014) considered USA outbreaks from melons (watermelon, honeydew, rockmelon) from 1973 – 2011 and noted that outbreaks were most often associated with rockmelons (85%), and suggested that it was due to the relatively neutral pH of rockmelons compared to watermelons and also the netted surface of rockmelons. Several reviews suggest that there are many sporadic cases of listeriosis that are *not* linked to 'outbreaks' and are less likely to be widely reported, and also that there are small listeriosis outbreaks from contaminated produce prepared in institutional (health care) or retail settings (Buchanan et al., 2017; Callejón et al., 2015; Desai et al., 2019). In those cases, the source of contamination is not necessarily due to

contaminated produce but may also arise from contamination in the food preparation environment. The most common agents of foodborne disease outbreaks from melons are *Salmonella* spp. followed by norovirus and then pathogenic *E. coli* (NSW Food Authority, 2013; FSANZ, 2011; Danyluk et al., 2014b; Callejón et al., 2015) with rare reports of illness from *Campylobacter* and *Cyclospora* (FSANZ, 2011). However, *listeriosis outbreaks from rockmelons are rare*, with only three outbreaks being reported internationally in the last 40 + years, i.e., the oldest data start in 1973 (Walsh et al., 2014).

Of those outbreaks, two occurred in Australia (in 2010 and 2018). The 2010 outbreak in Australia resulted in nine cases and two deaths (FSANZ, 2011). The 2018 outbreak in Australia involved 22 cases and caused seven deaths and one miscarriage (NSW DPI, 2018b). The other reported listeriosis outbreak involving rockmelons occurred in the USA in 2011 and led to 147 cases and 33 deaths (McCollum et al., 2013).

Curiously, there have been no reported listeriosis outbreaks from rockmelons in any countries other than USA/Canada and Australia and the vast majority of reported outbreaks from rockmelons (from all pathogens) are from USA/Canada, with very few reports of rockmelon-borne illness from Europe or other nations. For example, there were no reports of rockmelon-associated foodborne illness outbreaks (from any pathogen) in 37 European countries in 2017 (EFSA/ECDC, 2018) despite that it is also a popular fruit in Europe[5] and that per person consumption is similar to the USA. Most foodborne illness outbreaks from fruits in Europe are linked to berry fruits, while most in the USA have been linked to melons (Callejón et al., 2015): from 2004 – 2012 in USA 23% of 377 reported outbreaks from fresh produce were from fruits and, of those fruit-related outbreaks, 29% were from melons. Of the 198 reported produce related outbreaks in Europe during the same time interval, 33% were from fruits, but only 2% were from melons. Walsh et al. (2014) reported that, of rockmelon-related outbreaks in the USA, 69% were from melons imported to North America from Mexico and Central America.

5 The strain of rockmelon most cultivated in Europe is *Cucumis melo* var. *cantalupensis*, and is lightly ribbed compared to the strain *C. melo* var. *reticulatus* most consumed in USA and Australia. European consumption is estimated as 3.76 kg/person-year (https://www.freshplaza.com/article/2173931/europe-melon-market-trends/ accessed 1 Jan, 2020), while consumption in USA is estimated as 3.1 kg/person-year (https://www.statista.com/statistics/257220/per-capita-consumption-of-fresh-cantaloup-melons-in-the-us/ accessed 1 Jan, 2020).

Table 1. Outbreaks of *L. monocytogenes* and *Salmonella* associated with melons from 2010 to 2020

Year	Country	Pathogen Food	Cases *Hospitalised* (Deaths)	Probable causes	References
2019	USA (Multistate)	*Salmonella* Carrau Pre-cut melon	137 *38*	Not identified.	(CDC, 2019)
2018	Australia (Multistate)	*Listeria monocytogenes* Rockmelon	22 (8)	"*Twenty-two human cases of listeriosis occurred in Australia between January 16 and April 10, 2018. The strain of* Listeria monocytogenes *causing these infections was related by whole genome sequencing (phylogenetic analysis) to isolates recovered from samples taken from 37 rockmelons sourced from retail and wholesale supplied by the Farm, one isolate obtained from melons on farm, and one isolate from an environmental swab taken at the packhouse. This detailed laboratory testing information, combined with epidemiological analysis, purchase history and product trace back, indicate that rockmelon produced at the Farm is the source of infection for the people infected with this strain of* Listeria monocytogenes*."* (NSW DPI, 2018b). "*the operation was generally well run with no obvious hygiene concerns. An investigation found that the most likely cause of the outbreak was a heavy rainfall event and subsequent dust storms in the area which resulted in a higher than usual amount of bacteria on fruit. The washing systems utilised by the grower were consistent with good industry-practice, which cannot guarantee that all* Listeria monocytogenes *is removed from the surface of the fruit*." (NSW Food Authority, 2019).	(NSW Food Authority, 2019; NSW DPI, 2018b)
2018	USA (Multistate)	*Salmonella* Adelaide Pre-cut melon	77 *36*	Not identified.	(FDA, 2018)
2016	Australia (Multistate)	*Salmonella* Hvittingfoss Rockmelon	144	"*Outbreak contributing factors - No initial monitoring of chlorine-based sanitiser - Use of 10ppm stick to measure a sanitiser level of 100 ppm. General hygiene of facility - Excess dirt, dust - Recirculated water unit not adequately cleaned*", (NSW DPI, 2018a).	(FSANZ, 2016b; NSW DPI, 2018a; NSW Food Authority, 2017)

2012	Europe (Multi-national)	*Salmonella* Newport Watermelon (whole and cut)	63 *5* (2)	Not identified. Brazilian melons were implicated as the source. This outbreak potentially involved use of water contaminated by faecal residues of animals.	(EFSA/ECDC, 2018; EFSA/ECDC, 2014)
2012	USA (Multistate)	*Salmonella* Typhimurium and *Salmonella* Newport Rockmelon	261 *94* (3)	"*Based on the positive test results from the environmental samples collected from the Chamberlain Farms production fields and packinghouse during two separate FDA investigations, it is likely that the initial contamination of the melons occurred in the production fields and was spread by operations and practices within the packinghouse. It is also likely that the contamination proliferated during storage and transport to market*." (FDA, 2014). There were a number of factors, such as insufficient monitoring of sanitizers and the lack of control of drying and cooling, of melons that were suggested to, potentially, increase the level of contamination from the field (FDA, 2014).	(FDA, 2014; FDA, 2013; FDA, 2012b)
2011	USA (Multistate)	*Listeria monocytogenes* Rockmelon	147 *143* (33)	*"This team identified the following factors as those that most likely contributed to the introduction, spread and growth of Listeria monocytogenes in the cantaloupes: There could have been low level sporadic Listeria monocytogenes in the field where the cantaloupe were grown, which could have been introduced into the packing facility.* *A truck used to haul culled cantaloupe to a cattle operation was parked adjacent to the packing facility and could have introduced contamination into the facility. The packing facility's design allowed water to pool on the floor near equipment and employee walkways.* *The packing facility floor was constructed in a manner that made it difficult to clean. The packing equipment was not easily cleaned and sanitized; washing and drying equipment used for cantaloupe packing was designed for and previously used for postharvest handling of another raw agricultural commodity. There was no pre-cooling step to remove field heat from the cantaloupes before cold storage. As the cantaloupes cooled there may have been condensation that promoted the growth of Listeria monocytogenes".* (FDA, 2012a) "*In May of 2011 the Jensen brothers allegedly changed their cantaloupe cleaning system. The new system, built to clean potatoes, was installed, and was to include a catch pan to which a chlorine spray could be included to clean the fruit of bacteria. The chlorine spray, however, was never used*." (US Department of Justice, 2013)	(FDA, 2011b; FDA, 2012a; CDC, 2011a; CDC, 2011b; CDC, 2012; US Department of Justice, 2013; FDA, 2011a)
2011	USA (Multistate)	*Salmonella* Panama	20 *3*	Not identified. Melons implicated in the outbreak were sourced from Guatemala.	(CDC, 2011c)

		Rockmelon			
2010	Australia (Multistate)	*Listeria monocytogenes* Melons and/or melons contained within fruit salads	9 *9* (2)	"*Unfortunately, no data or observations are available that provide details of the possible mechanisms of melon contamination that led to the 2010 L. monocytogenes outbreak in New South Wales (NSW), Victoria and Queensland."* (FSANZ, 2011).	(FSANZ, 2011; OzFoodNet, 2010b; OZFoodNet, 2010a)
Pre-2010 Australian outbreaks					
2006	Australia	*Salmonella spp.* Rockmelon	100 *9*	"*Salmonella* Saintpaul *was microbiologically linked to rockmelons grown and processed in the Northern Territory (NT); rockmelons from Queensland were found to be contaminated with non-outbreak associated strains of Salmonella spp.. The outbreak strain could not be definitively linked to a farm, packing shed or processor, however, investigations of six processors in the NT and Queensland identified critical food safety issues in the production and processing of rockmelons that may have contributed to produce contamination; including the use of untreated or inadequately treated water on ready-to-eat melons, the incorrect use of disinfectants, temperature differential between fruit and wash water and processing of damaged fruit (*Munnoch *et al.,* 2009*)*." (FSANZ, 2011).	(FSANZ, 2011)

6.1 Key findings and outcomes from *L. monocytogenes* outbreak investigations

Due to the seriousness of disease outbreaks from rockmelons, outbreak investigations have been conducted and findings have been published and summarised (Callejón et al., 2015; FAO/WHO, 2011; NSW Food Authority, 2014; Walsh et al., 2014). In general, the causes of outbreaks from rockmelon (i.e., from contamination with *Salmonella*, norovirus or *L. monocytogenes*) differ. Both *Salmonella* and norovirus cause gastrointestinal illness and are spread to foods *via* faecal contamination. *Salmonella* outbreaks from rockmelons are believed most often to arise from contamination in the field from contaminated (irrigation) water, adverse weather events carrying contaminated water to growing areas, soil, animal faeces (e.g., birds, reptiles, mammals) and including application of incompletely composted organic fertilisers. These factors can be exacerbated by lack of temperature control and residual moisture on the surfaces of melons, allowing the pathogens to grow during storage and distribution, but can also be spread by infected food handlers (Walsh et al., 2014; Gagliardi et al., 2003; FDA, 2014; FDA, 2013; FAO/WHO, 2011; Bowen et al., 2006). Norovirus is a human-specific pathogen and is transmitted by the 'faecal-oral' route. Foodborne outbreaks are due to contamination from infected food handlers, or could arise, e.g., from irrigation water contaminated with human faeces from an infected person. Listeriosis, however, is a systemic infection and does not usually cause gastrointestinal infection and is generally not thought to be transmitted by the faecal oral route.

Thus, the causes of *L. monocytogenes* outbreaks related to rockmelons are thought to be different and consistent with the ecology of *L. monocytogenes* in ready-to-eat foods and food processing environments, in that it can lead to colonisation of factories which then contaminates foods processed in those facilities. As such, causes of non-*Listeria* outbreaks (*Salmonella*, norovirus, *E. coli)* from rockmelon are generally different than those from the *L. monocytogenes* outbreaks, and consideration of causes of outbreaks from *Salmonella*, or *E. coli* or norovirus do not greatly contribute to our understanding of causes (and relevant risk management) of *L. monocytogenes* outbreaks from rockmelons except in terms of managing plant hygiene generally, and minimising the opportunity for growth of the melon surface by keeping it dry. Another key difference is that growth of *Salmonella* and *E. coli* can be prevented by maintaining temperatures at <5°C, but this is ineffective against *L. monocytogenes* (*see* Section 3.1).

6.2 *L. monocytogenes* outbreaks from rockmelons

As discussed earlier (see Section 3), *L. monocytogenes* is known to colonise food processing factories and to require specific management in food plants that produce ready-to-eat foods. Rockmelons, as fruits, are not considered to be 'ready-to-eat' foods under the Australian Food Standards Code (FSANZ 2016b) which states that "ready-to-eat food means food that is ordinarily consumed in the same state as that in which it is sold and does not include nuts in the shell and whole, raw fruits and vegetables that are intended for hulling, peeling or washing by the consumer". However, as discussed in Section 9, it appears that *L. monocytogenes* can grow on the surface of rockmelons and, while the rind of the rockmelon is not consumed, the act of cutting the rockmelon can transfer contamination to the edible portion of the fruit which also strongly supports *L. monocytogenes* growth. As such, management of *L. monocytogenes* risk from rockmelons has many parallels with management of the risk from traditional 'ready-to-eat' foods.

While there are only three known outbreaks of listeriosis from whole rockmelons, the consequences of those outbreaks have been severe for both consumers and the industry both in the USA and Australia (NSW DPI, 2018b; McCollum et al., 2013) both personally and financially. After the public declaration of the 2018 Australian rockmelon outbreak, demand for rockmelons in Australia was reported to have declined by 90% (ABC, 2018). Many small Australian producers have left the industry (J. Caleo, pers. comm., 2019) and the overall cost to the Australian industry was estimated at around $60 million by the then Australian Federal Agriculture minister, David Littleproud (Courier Mail Newspaper, 2018).

The low incidence of *L. monocytogenes* outbreaks from rockmelons means that there are few detailed investigations of the causes of outbreaks. The 2010 Australian outbreak investigation found only that the rockmelons implicated in that outbreak were produced in the NSW (Griffith) growing area (FSANZ, 2011). While no direct link was able to be made between positive samples of rockmelons contaminated with the outbreak strain and the rockmelons that the people who became ill had eaten, OzFoodNet (2010b) concluded that there was strong epidemiological evidence that "rockmelon and/or honeydew melon, eaten fresh or used in the preparation of fruit salads" was the source. As noted in Table 1, the mechanism of contamination of the melons could not be determined (FSANZ, 2011) and the 2010 outbreak is not considered further in this Section. Similarly, the investigation of the 2018 outbreak in Australia (NSW DPI, 2018b) was not able to find

definitive causes for the outbreak, but suggests that environmental contamination from weather events increased the microbial load on fruit which could not be minimised with washing and sanitation processes in place. It is noteworthy that the investigation found no evidence of the *L. monocytogenes* outbreak strain in the growing environment (which is generally consistent with the observations of Vivant et al. (2013) – *see* Section 3), and only one isolate of the outbreak strain was found in the implicated packhouse from a boot swab in the processing area, and from one composite swab of five washed melons at the packhouse.

However, a survey of the suspected product at retail in Australia (NSW DPI, 2018b) found that >90% of more than 35 rockmelons from the suspected producer that were purchased at retail or wholesale, over several days and across two states, were positive for the outbreak strain, suggesting that the cantaloupes from that producer were systematically contaminated over time from an unknown source or process[6]. Similarly, in the investigation of the 2011 USA outbreak, while no outbreak strains were found in the growing environment or on melons in the field, five of ten rockmelons sampled from the cool room of the packhouse were contaminated with one of the outbreak strains and, of 18 rockmelons purchased from retail stores, 17 were contaminated with one of the outbreak strains (McCollum et al., 2013). The FDA investigation revealed that five distinct strains of *L. monocytogenes* caused the outbreak, and that each strain was found in the pack-house environment in wet areas.

As noted, the most extensive investigation of a rockmelon-related listeriosis outbreak was the Jensen Farms outbreak in the USA in 2011 (McCollum et al., 2013; FDA, 2011b). The FDA's Coordinated Outbreak Response and Evaluation Network (CORE) (FDA, 2012a), stated that the following likely contributed to the contamination:

- A truck used to haul culled cantaloupe to a cattle operation was parked adjacent to the packing facility and could have introduced contamination into the facility.
- The packing facility's design allowed water to pool on the floor near equipment and employee walkways.

6 WHO (2018) identified that product from the implicated farm was exported to nine other countries. At about that time there were listeriosis cases linked to rockmelon in Singapore which was one of the countries to which the implicated Australian melons were exported. However, the Singapore cases did not involve the strain that led to the 2018 Australian outbreak (ProMED, 2018).

- The packing facility floor was constructed in a manner that made it difficult to clean.
- The packing equipment was not easily cleaned and sanitized; washing and drying equipment used for cantaloupe packing was designed for and previously used for postharvest handling of another raw agricultural commodity.
- There was no pre-cooling step to remove field heat from the cantaloupes before cold storage. As the cantaloupes cooled there may have been condensation that promoted the growth of *Listeria monocytogenes*.

Furthermore, it was later reported that Jensen Farms failed to use a chlorine spray on the melons (US Department of Justice, 2013).

Those observations underpinned a series of recommendations by FDA (2011c), i.e.,:

- Assess produce facility and equipment design to ensure adequately cleanable surfaces and eliminate opportunities for introduction, growth, and spread of *L. monocytogenes* and other pathogens.
- Assess and minimize opportunities for introduction of *L. monocytogenes* and other pathogens in packing facilities.
- Implement cleaning and sanitizing procedures and verify the efficacy of cleaning and sanitizing procedures.
- Periodically evaluate the processes and equipment used in packing facilities to assure they do not contribute to fresh produce contamination.

McCollum et al. (2013), in their analysis of the outbreak, emphasised that fresh-produce processors should consistently use good agricultural and manufacturing practices ('GAP' and 'GMP') to minimise the possibility of foodborne-pathogen introduction and food contamination. In other fresh produce-associated listeriosis outbreaks the production/processing environment has also been implicated as the source of contamination rather than the growing/harvesting environment (Garner and Kathariou, 2016).

From the Australian 2018 outbreak investigation, NSW DPI (2018b) recommended that:

a. All growers should review the entirety of their operations and food safety plans as a matter of priority. These plans need to ensure that external environmental factors are taken into account (e.g., adverse weather events, propensity for soil to adhere to rockmelon surfaces) and that processing/packing operations are able to adjust or cope with significant variations in soil/bacterial load.

b. All wash steps should be reviewed …. to ensure best practice is utilised across the industry as a whole. This review should include the in-house expertise and understanding of growers and staff, that adequate sanitisers are being applied and at an appropriate concentration and contact time, depending on conditions.

c. Aspects of washing systems should also be reviewed to ensure the adequacy of the sanitiser type, the effectiveness in the number of spray heads in sanitiser tanks and their design, that immersion tanks have sufficient available free sanitiser concentration, and that recirculated water is dumped at appropriate intervals.

d. Cleaning and sanitising procedures within the general shed environment need to include steps for:

    - regular removal and cleaning of dusty surfaces at all levels in the packhouse;
    - water treatment to render water potable for cleaning and hand washing;
    - cleaning and sanitising harvest equipment and tools;
    - cleaning and sanitising cleaning implements;
    - no high-pressure cleaning in the packing shed;

e. Growers should also consider implementation of Standard Plate Count or other testing on fruit as an early indicator that higher bacterial loads are present, requiring additional control measures (such as increased sanitiser strength during washing).

6.3 Conclusions

In the rare cases of listeriosis outbreaks from rockmelons, they appear to arise from sporadic contamination events in the field, or other sources, leading to contamination and colonisation of

the packhouse by *L. monocytogenes*, rather than extensive contamination in the field. Colonisation by *L. monocytogenes* of food processing factories that produce/process ready-to-eat foods is well described in the scientific literature and methods for control are now well-established in the dairy, smallgoods and lightly-preserved fish processing industries. The review of Garner and Kathariou (2016) also concluded that listeriosis outbreaks from fresh produce could often be traced to the same sources as in outbreaks associated with other food vehicles, i.e., the processing plant and equipment. While not explicitly discussed in the investigation report of the 2018 Australian outbreak (NSW DPI, 2018b), the very high prevalence of the outbreak strain of *L. monocytogenes* on fruit sampled at wholesale and retail suggests that contamination of the fruit released for sale could have also arisen in the packhouse, e.g., that colonisation of the packhouse after an adverse weather event led to contamination of fruit in the field that was transferred to the packhouse or, as discussed , that high prevalence, but low level, contamination occurred in the field after adverse weather events and was not reliably eliminated by the sanitisation step in the packhouse (Australia 2018 outbreak).

Because *L. monocytogenes* can grow on the surface of rockmelons (*see* Section 9.2) the lessons learned from, and risk management strategies implemented in, industries that produce 'ready-to-eat' foods can inform *L. monocytogenes* risk management in the rockmelon industry, particularly concerning the relevance and conduct of (in-plant) environmental monitoring procedures.

The review indicated that the rare outbreaks of listeriosis from rockmelons seem to be associated with a change in conditions in the field or the packhouse that introduce and/or allows colonisation by the pathogen. If contaminated melons from the field pass through or overwhelm the sanitising systems and there is no environmental monitoring or if reliable cleaning regimes are not implemented*, L. monocytogenes* can then colonise the packhouse and contaminate even 'clean' melons.

As such, we recommend continued development and communication of wholistic risk management strategies that includes growers assessing and responding to adverse weather events, or other unusual circumstances, monitoring of the potential for packhouse colonisation, and more effective and reliable handling of fruit from the field and during processing and transport to minimise the potential for *L. monocytogenes* growth.

7 Review of previous 'Best Practice Recommendations' provided to the melon industry

The objective of this review is to identify 'Best Practice Recommendations', provided or endorsed by authoritative bodies, that have previously been provided to the rockmelon industry covering primary production to when melons leave the farm gate, both in Australia and internationally and to comment on key similarities or difference relevant to the reduction of contamination by *L. monocytogenes.*

Five best practice guides specific for melon production were identified in the literature review for food safety control during growing, harvesting and processing[7] of melons (four from the USA, one from Australia). These are:

- California Commodity Specific Food Safety Guidelines for the Production, Harvest, Cooling, Packing, Storage and Transporting of Cantaloupes and other Netted Melons (PMA/WG, 2013);
- Commodity-Specific Food Safety Guidelines for the Eastern Cantaloupes Growers Association (ECGA, 2013);
- National Commodity-Specific Food Safety Guidelines for Cantaloupes and Netted Melons (PMA/WG/UFPA/FPAA, 2013);
- Commodity Specific Food Safety Guidelines for the Melon Supply Chain (PMA/UFFA, 2005); and
- Melon Food Safety: A Best Practice Guide for Rockmelons and Specialty Melons (NSW DPI, 2019b), which represents the most relevant and recent comprehensive advice provided to the Australian industry

Other relevant documents identified in the review process included FAO/WHO (2008), EFSA (2017) and Hurst (2017) and discuss good agricultural practices and risk mitigations consistent with the above 'best practice' guides. The Codex Alimentarius Commission (CAC) has specifically discussed

7 Processing in USA Guidelines refers to fresh-cut processing facilities; in Australian guidelines the term refers to packhouse processing, where no transformation of the fruit occurs, otherwise the term fresh-cuts is used.

microbial hazards for melons and potential interventions (FAO/WHO, 2011). In some instances, advice from Australian State Government organisations has also been provided (Agriculture Victoria, 1995; DAF QLD, 2016; NT GOV, 2019).

The following summarises the recommendations of the five main guidelines documents mentioned above. Details vary but it is recommended that pre- and post-harvest practices should minimise stem scar and rind internalisation of pathogens (*see* Section 9.3) into the edible portions of the melon flesh (PMA/UFFA, 2005). It is worth noting that these best practice documents provide general recommendations for all pathogens, not only *L. monocytogenes* and as such, are not specific for the management of *L. monocytogenes* in rockmelons. Currently, the only *Listeria* specific guidance is "Guidance on Environmental Monitoring and Control of *Listeria* for the Fresh Produce Industry" (UFPA, 2018), but is not specific for the melon industry.

7.1 Pre-harvest risk management recommendations

Key issues identified in guideline documents include domestic and wild animal exclusion, irrigation and fruit contact spray water, weather events (flooding, heavy rains, prevailing winds), land history, urban encroachment, field hygiene (worker personal hygiene, waste removal and provision of toilet facilities), and soil amendments.

Guidelines documents recommend that an environmental risk assessment, that includes the factors listed above, should be conducted pre-planting and within one week of harvest to consider risks from the soil, initially, and factors that may have affected product safety during growing.

It is recommended that domestic animals, wildlife, insects, and pests be controlled, reduced or eliminated and that, if there is evidence of animal damage or faecal contamination, harvest should be postponed (PMA/WG, 2013), and the affected crop area isolated and the produce destroyed (PMA/WG/UFPA/FPAA, 2013; ECGA, 2013).

Irrigation and spray water are considered a primary source of pathogens and water quality acceptability depends on the intended use of the water. In the USA, acceptance criteria are based on the accumulation of historical data and testing at least monthly during the growing season. The FDA Produce Safety Rule (PSR) (FDA, 2015b) provides contemporary guidance. For water that

comes into contact with the harvestable portion of the crop, a geometric mean (GM) of <126 CFU generic *E. coli* and a statistical threshold value (STV) of <410 generic *E. coli* per 100 ml of water. The number of water samples included in the GM depend upon the water source: at least 20 samples for untreated surface water (collected over 2-4 years initially, then with at least 5 samples annually after the first 20 samples are collected), and 4 samples for untreated ground water (collected over 1 initially, then with 1 sample annually over the subsequent years). If water exceeds these criteria Corrective Measures (including water treatment, commercial washing, and die-off intervals), provide additional ways water can continue to be used. At least annually, farms must inspect the agricultural water system to identify conditions that are reasonably likely to introduce hazards into or into produce, and must maintain the water distribution system to prevent and maintain it from serving as a source of contamination. Water used for hand washing, cleaning and sanitizing food contact surfaces, and in any postharvest applications should have >1 generic *E. coli* /100 ml; untreated surface water cannot be used.

In Australia, water in direct contact with rockmelons, including water used for agricultural chemical application, must not have ≥ 10 CFU/100mL thermotolerant coliforms nor ≥1 *E. coli* CFU/100mL. If drip irrigation is used the criterion is < 1000 CFU/100mL thermotolerant coliforms (NSW DPI, 2019b). Testing is required at least annually. These water quality requirements are consistent with the Australian Guidelines for Fresh Produce Food Safety (Fresh Produce Safety Centre ANZ, 2019). In addition, water used for handwashing must not contain ≥ 1 CFU/100mL *E. coli*. Water pooling should be prevented in growing fields and use of drip irrigation is preferred. Notably, most Australian growers now use under-ground drip irrigation. Irrigation water quality guidelines are summarised in Table 2.

Some recommendations advise that if flooding or heavy rains occurs harvest should be postponed, and consideration given to extra postharvest washing. In that case, all rockmelons that floodwater has touched, either in the field or stored in bulk, are considered adulterated and should not be used for human or animal food. Queensland Government guidelines also recommend additional sanitation, or disposal, of fruit (including rockmelons) that have been exposed to flood waters (DAF QLD, 2016). ECGA (2013) state that cantaloupes must not be planted in formerly flooded ground for at least 60 days or unless soil acceptance criteria for *Salmonella*, enterohaemorrhagic *E. coli* (EHEC) and enterotoxigenic *E. coli* (ETEC) of < 1/30g are met. Buffer zones from flooding (ECGA, 2013), animal ingress and prevailing winds that could carry pathogens (NSW DPI, 2019b)

should be used. In the USA, cantaloupes must not be harvested within 30 ft (9m) of floodwater (ECGA, 2013)[8].

Table 2. Irrigation water quality guidelines

Water use	Microbiological acceptance criteria	Reference
Direct product contact	*E. coli* < 126 CFU/100mL (geometric mean of 20 samples for surface water over previous 2-4 years OR 4 samples of ground water from previous 1-4 years) and < 410 CFU/100mL (statistical threshold value calculated from same samples as above)	PSR USA
	Thermotolerant coliforms < 10 CFU/100mL and *E. coli* <1 CFU/100mL	Australia
Non-direct fruit contact	No criteria	PSR USA
Drip irrigation	Thermotolerant coliforms < 1000 CFU/100mL	Australia

PSR = FDA Produce Safety Rule (FDA, 2015b)

Assessment of previous land use and adjacent properties for contamination sources e.g., hazardous waste sites, feedlots, domestic animals or sewage from humans and urban development should be conducted. If risk mitigation cannot be achieved, it is recommended that melons are not marketed without further processing or that the potentially contaminated crop is eliminated (PMA/WG, 2013) and that if adjacent land use includes i) composting operations, concentrated animal feeding operations or non-synthetic soil amendment stockpiles, or ii) grazing lands, domestic animals, or potential septic leachates, melons should not be grown within 122m and 9m respectively (PMA/WG, 2013).

Damaged fruit should be removed to prevent waste build-up and pest attraction, and employees should be adequately trained to recognise these risks, which can increase with multiple harvests.

8 The 9 m rule is apparently derived not from scientific studies, but from the distance required to turn a typical tractor, i.e., the rule is intended to stop the tractor tyres from transferring contaminated water or mud into the growing areas, or from splashing from tyres.

Minimisation of ground spots on melons and the use of plastic mulch (NSW DPI, 2019b) or cups that are sanitised (PMA/WG, 2013; PMA/WG/UFPA/FPAA, 2013; ECGA, 2013) is also recommended.

Table 3. USA guidelines for best practice use of soil amendments for melon production.

Soil amendment	Use	Microbial specification
Raw manure, biosolids, incompletely composted green waste	Wait 12 months prior to planting (PMA & WG 2013) Wait 2 years prior to planting (ECGA 2013)	Not applicable
Composted animal products (PMA & WG 2013)	> 45 day withholding period before harvest	Faecal coliforms <100 MPN/g *Salmonella* <1/100g *E. coli* O157:H7 <1/100g
Physically heat-treated animal products	If validated, no withholding period If unvalidated, > 45 day withholding period before harvest	Faecal coliforms <100 MPN/g *Salmonella* <1/100g *E. coli* O157:H7 <1/100g
Composted animal products (ECGA 2013)	> 45 day withholding period before harvest	Faecal coliforms <1000 MPN/g *Salmonella* <1/30g *E. coli* O157:H7 <1/30g *L. monocytogenes* <1/30g
Physically heat-treated animal products (ECGA 2013)	If validated, no withholding period If unvalidated, 45 day withholding period before harvest	Faecal coliforms <10 MPN/g *Salmonella* <1/30g *E. coli* O157:H7 <1/30g

NW DPI (2019b) recommends that raw animal manures should not be added to soils used for melon production and treated manures containing animal manures or poultry litter should be avoided. National and/or state guideline documents/standards for the treatment of composted soil amendments have been developed. Regulatory compost standards in the USA (FDA, 2019a)

and Australia (Standards Australia, 2012) are similar, but some recommended microbiological criteria in the USA specified by auditors/buyers are more stringent than Australia (*see* Table 3). In Australia all composts, soil conditioners, and mulches should not exceed 1000 MPN/g faecal coliforms while *Salmonella* should be absent in 50g (Standards Australia, 2012).

An exclusion period of 90 days between use of untreated manure and harvest is specified for rockmelons in Australian guidelines (Fresh Produce Safety Centre ANZ, 2019) although the use of untreated manure is not allowed according to NSW DPI (2019b). For treated soil amendments an exclusion period of 45 days is recommended (Fresh Produce Safety Centre ANZ, 2019).

7.2 Harvest risk management recommendations

Key risk management issues that have been identified in relation to harvest include equipment cleaning, people, transportation and mechanical damage.

For best practice it is generally recommended that:

- Holding time prior to pre-cooling/cooling should be minimised to prevent microbial growth on melons.
- Sanitary stations and field toilets with water, soap and single use towels should be provided and their usage by field staff ensured.
- Staff be trained to prevent fruit damage, recognize food safety risks including damaged fruit or presence of animal faeces, and recognise any changes since pre-harvest assessment (PMA/WG, 2013).
- Rough handling should be avoided and damage from equipment and tools, particularly at the stem end, minimised.
- Dropped melons must not be harvested.

Multiple harvests increase the potential for pathogen introduction, so the following recommended practices are considered important for microbial food safety management of rockmelons:

- Over-ripe, damaged and diseased fruit should be culled to a designated waste area. An aggressive melon cull disposal and waste removal program should be implemented to limit field, packinghouse, and cooler culls and thus reduce the potential for insect to melon fruit contamination (PMA/UFFA, 2005).

- Equipment should be cleaned and sanitised after each shift and all equipment and tools maintained in good condition.

- Equipment that is (i) dedicated to harvesting and (ii) readily cleanable should be used. Seasonal use of harvest equipment introduces risk from pest infestations and dirt build-up. Equipment sanitation procedures must ensure microbial loads are lowered and validation of procedures may be required (PMA/UFFA, 2005).

- Contamination from transport vehicles to people, equipment, soil and water (PMA/WG/UFPA/FPAA, 2013) should be prevented and equipment used in high-risk operations (e.g. compost transfer) segregated.

- Potential for soil to melon contamination should be minimised. When removing soil from melons, cloths should not be used, and cross-contamination should be prevented by other means. e.g. use of dry dumps.

NSW DPI (2019b) recommends against field packing of melons, specifying they must be pre-cooled, washed and sanitised before distribution. However, some low volume field packing still occurs in Australia, where melons from some growers are packed as received without a wash step.

In addition to the best practices outlined above, field packing best practices in the USA (ECGA, 2013) include that:

- a responsible person is designated for harvesting.

- containers are inspected for pest contamination.

- corrugated containers and containers made of porous materials are prohibited from use.

- field and finished product containers are distinguished (e.g., colour coding).

- containers that have been in direct contact with the soil are not stacked.

All guidelines recommend irrigation should cease prior to harvest within a time frame to prevent mud and soil contamination on the melons. Australian guidelines specify an exclusion period of 48 hours if the water contains *E. coli* > 100 CFU/100mL (Fresh Produce Safety Centre ANZ, 2019). Water used during harvest should meet microbiological criteria of generic *E. coli* < 1 CFU/100mL (Fresh Produce Safety Centre ANZ, 2019), 0 CFU/100mL (ECGA, 2013) or < 2 MPN /100mL and >10ppm free chlorine, pH 6.5-7 or >725mV ORP (PMA/WG, 2013) and compliance with these criteria should be monitored. The water should be tested prior to harvest and monthly during harvest.

7.3 Post-harvest risk management recommendations

The key issues identified in all the Guidelines documents relevant to post-harvest handling and packhouse processing include dumping, cooling and cold storage of melons, wash water sanitation, fungicide treatments, people, equipment cleaning, building hygiene and transportation.

For best practice, it is generally recommended that:

- Dry dumping is preferred over wet dumping, although it can result in more damage to fruit.
- If wet dumping is used, water should be of drinking water quality, disinfected, and sanitiser level, and fruit and water temperature, regularly monitored. Single use water is preferable but if recycled, the frequency of change should be determined by objective measurements (e.g. turbidity) (NSW DPI, 2019b). Throughput time in dump water should be known and minimised. It is recommended that dump tanks are emptied, cleaned and refilled daily (ECGA, 2013). All best practice documents discuss the risk of pathogen infiltration if melons are warmer than dump water, but only (NSW DPI, 2019b) specifies that dump tank water should be at least 5°C warmer than fruit pulp temperature.
- A rinse step using sanitised water should follow the wet dump tank, as applicable.
- Melons should be pre-cooled as rapidly as possible to the industry temperature standard with (NSW DPI, 2019b) specifying 5-8°C. PMA/UFFA (2005) assert “cooling, cold storage and refrigerated distribution/marketing of whole melons as raw agricultural commodities

is not required to maintain the safety of whole melons", but we note that this advice predates all listeriosis outbreaks from rockmelons internationally and is superseded by recommendations after the USA 2011 outbreak which implicated the potential for growth on the surface of rockmelons when fruit temperature was not reduced and/or where condensation could develop on the surface of the rockmelon (*see* Section 6.2). Rather, based on those later outbreaks, the prevention of free moisture on the surface of melons during cooling or storage is considered important to prevent microbial growth.

- Standard cool rooms are ineffective for cooling melons rapidly. Forced air cooling presents a lower risk and is preferred over water cooling, but equipment cleanliness must be maintained. Ice for cooling purposes is to be avoided because of the risk from cross-contamination. Condensation from cooling systems must run directly to drainage systems and not drip onto melons. Separate cool rooms are to be used for pre- and post-processed fruit, with stacking organized to allow for adequate airflow and even temperature distribution.

- All dumping equipment, cooling equipment, cold rooms, and transport vehicles are to be cleaned and sanitised to prevent cross-contamination. (n.b., cleaning frequencies are not specified, rather they are recommended to be set within the context of the entire food safety management system of the business.)

- Drinking quality water must be used for all postharvest operations and meet relevant criteria (Table 4), noting that there is inconsistency in the stringency of those criteria between guidelines documents.

The primary purpose of sanitiser use in postharvest water is to prevent the water from becoming contaminated should pathogens be introduced into the water from melons, i.e., it is possible that the contaminated water could then act as a source of contamination of incoming melons.

All best practice documents recommend continuous monitoring of water sanitiser levels, that water treatment systems are actively maintained, cleaned and calibrated and that the water treatment used should be validated and regularly verified half-hourly (NSW DPI, 2019b) to hourly (PMA/WG, 2013).

Table 4. Proposed postharvest water quality criteria for melon processing.

Source	Microbiological Criterion
(NSW DPI, 2019b)	*E. coli* < 1 CFU/100mL
(ECGA, 2013)	Total coliforms not detected in 100mL
(PMA/WG, 2013)	Total coliforms not detected in 1L[9]

A single use wash water system is preferred but if water is recycled it is recommended that it should be treated to maintain drinking quality. Water chemistry (e.g. pH, hardness, turbidity), sanitiser level and contact time must be monitored and recorded to maintain efficacy of washing sanitation.

NSW DPI (2019b) specifies wash water treatments and that fruit pulp temperature should be at least 5˚C cooler than wash water (Table 5). USA best practice advises that sanitisers be used according to the manufacturer's specifications. PMA/WG (2013) specify wash treatment to include chlorine at > 10 ppm at pH 6.5-7.0 or > 725 ORP when single pass water from a spray bar is used. Postharvest fungicide treatment should be a separate step to washing, using drinking quality water for the fungicide application. Best practice is spray application with no water recycling, and hot water treatment (50-55°C) is more effective (NSW DPI, 2019b). If hot water is used as an alternative fungicide treatment the contact time and temperature should be validated (ECGA, 2013; PMA/WG/UFPA/FPAA, 2013) and monitored (PMA/UFFA, 2005).

Employees and visitors to packhouses should receive basic training in food safety risks, handwashing, glove usage, personal hygiene, and health reporting requirements. Personal hygiene requirements include clean clothing, use of protective clothing, consumption of water not to be allowed in production areas, and any other practices that prevent fruit contamination. Health regulations require that cuts and wounds on hands are to be disinfected and covered with water-proof dressings; local law requirements for notification of infectious disease should be adhered to, and anyone displaying symptoms of illness should be prohibited from contacting melons.

9 To date, we have found no scientific explanation for this very stringent criterion.

Table 5. Wash water treatments specified by NSW DPI (2019b).

Sanitiser	Target level	Contact time
Chlorine	200 ppm (pH 6.5-7.0) 100 ppm (measured as free chlorine)	1min 2min
Chlorine dioxide	5 ppm	2min
Peracetic acid	80 ppm	2min
Ozone spray	2-3 ppm (manufacturer's recommendation)	-
Bromo-chloro-dimethylhydantoin (Nylate)	5-10 ppm (pH 7.0-8.5) (manufacturer's recommendation)	-

Guidelines stipulate the appropriate number and location of toilet and handwashing facilities equipped with potable water, paper towels and hand sanitisers, and that hand sanitiser should not replace hand washing (PMA/WG/UFPA/FPAA, 2013). Doors from toilet facilities should not open directly to production areas (ECGA, 2013). Areas surrounding the packing facility should be kept free of waste, cleared to prevent pest harbourage and sealed to prevent dust and soil entering the facility.

The packhouse layout should prevent cross-contamination, pest infestation, minimise airborne contamination, and floors should be designed to facilitate adequate drainage. The facility should be fully enclosed and include zoning for low and high-risk areas. Floors should be kept as dry as possible and floor splash to equipment prevented. The facility and equipment should be kept in good repair to prevent contamination, and food contact surfaces should be constructed of material that prevents fruit damage.

All best practice guideline documents highlight the potential of high-risk items of equipment like roller brushes and cracked flaps or matting to act as harbourage sites for pathogens and to contaminate melons intermittently. It is recommended that unused equipment should be removed.

Facility and equipment sanitation practices should be implemented in accordance with standard GMP and GHP to prevent contamination and cross-contamination of melons. This should include:

- a master sanitation schedule
- pre-operation inspections
- use of materials that are easily cleaned and sanitised
- daily cleaning of the facility, including sanitising of food contact surfaces
- equipment specific SSOPs
- storage of hand-held tools in sanitiser solution
- appropriate cleaning techniques to prevent biofilm formation, and
- environmental testing to verify cleaning that includes testing for *Listeria*.
- the use of high-pressure hoses should be avoided (to minimise splashing from floors on to product). Employees must not walk or stand on food contact surfaces (PMA/UFFA, 2005).

Seasonal use of packing equipment introduces risk from pest infestations and dirt build-up. Equipment sanitation procedures must ensure microbial loads are lowered and validation of procedures may be required (PMA/UFFA, 2005).

The industry temperature standard should be used for transport in cleaned and sanitised vehicles. The guideline storage and transport conditions for rockmelons in Australia are 5°C at 90-95% relative humidity (NSW DPI, 2019b). In the USA, 2-5 °C at 95% relative humidity is typically used. Stacking must allow for adequate air flow to ensure even temperature distribution. Prevention of the formation of condensation on melons would also minimise potential for growth of *Listeria*, if present (*see* Section 3.1 and 9.2).

7.4 Comments on best practice recommendations in Australia and internationally for post-harvest treatment of whole melons with sanitisers

As noted above, different recommendations are provided in the various guidelines for the use of sanitisers. Historically, sanitisers in fresh produce processing have been used to prevent processing wash water from becoming persistently contaminated with pathogens from the produce and then cross-contaminating the incoming produce. However, sanitisers are increasingly being employed for their role in inactivating pathogens from the surface of produce and are being used in spray systems.

A key difference between the best practice recommendations provided for the sanitisation of melons by the NSW DPI (2109b) compared to the USA guidelines documents is that while the US documents suggest that users following the manufacturer's instructions, NSW DPI (2109b) recommends longer contact times than labeled by manufacturers. This difference is because the NSW DPI (2019b) suggestions are based on scientific research that specifically assessed the reductions of pathogens on the surface of rockmelons. It is important for growers and packhouse operators to understand that the manufacturer recommendations for the use of sanitisers may not be based on the assessment of their efficacy for reducing specific pathogens on their specific products, rather than being aimed at keeping wash water adequately sanitised and also not exceeding maximum residue limits on fruit. Therefore, a different contact time than labelled by the manufacturer may be required to achieve the desired risk reduction in *L. monocytogenes* on whole melon surfaces.

The publications "Melon Food Safety: A Best Practice Guide for Rockmelons and Specialty Melons" (NSW DPI, 2019b) and "Melon food safety toolbox: Practical resources for implementing best practice"(NSW DPI, 2019a), prepared by Dr Sukhvinder Pal (SP) Singh from NSW Department of Primary Industries, represent the most relevant and recent comprehensive best practice advice provided to the Australian melon industry and should be reviewed by all stakeholders.

8 Review of pre-harvest and post-harvest interventions for reducing the risk of *L. monocytogenes* on whole melons

The objective of this review was to communicate relevant authoritative research that describes the effectiveness of different interventions to minimise/control *L. monocytogenes* on whole melons at stages from primary production to when melons leave the farm gate. Where a publication provided data for pathogens in addition to *L. monocytogenes*, they were also reported.

8.1 Pre-harvest and harvest interventions for reducing the risk of *L. monocytogenes* on whole melons

Rockmelon production fields and surrounding environments can be a source of *L. monocytogenes* on rockmelons. Therefore, risk management may also be assisted by interventions that may be applied *before* harvest and processing. However, there are few studies and evidence for the effectiveness of pre-harvest or harvest interventions specifically for *L. monocytogenes* in the published literature.

Two papers reporting on potential interventions that could be applied pre-harvest or at harvest to reduce the risk from *L. monocytogenes* were identified. They are summarised below.

8.1.1 In field stem scar injections and spray application of levulinic acid and sodium dodecyl sulfate

Webb et al. (2015) reported on the efficacy of in-field stem scar injections of 200µl of 7.5% levulinic acid (LVA) with 1.0% sodium dodecyl sulfate (SDS), followed by a spray of 30ml 7.5% LVA with 0.5% SDS to prevent contamination after harvest through transport to the packing shed. Melons held at 22˚C for 24h after harvest were injected at the stem scar and spray treated over the entire melon surface. Contamination was simulated by either spot inoculating 11cm^2 sections of melon rind or stem scars with a three-strain *L. monocytogenes* cocktail. The melons were held for 2h at 22˚C then treated with either 200ppm chlorine or 5% LVA + 2% SDS in a dump tank simulation for 10min. The authors compared the results of spot inoculated melons that were field treated, with the results of a similar study that had slightly different dump tank times of 8min (Table 6). The results indicated that on the rind, pre-treatment in the field increased the $\log_{10}$CFU reductions of *L. monocytogenes* on whole melons following dump tank sanitisation. However, in this study injection and spraying were not tested separately, therefore it is not possible to

determine if one or both of these methods contributed to the increased reduction of *L. monocytogenes*. Moreover, contamination may occur prior to the injection and spraying of melons at harvest, and the efficacy of the treatment under this condition was not assessed. However, it is likely that the labour required to undertake injections of individual melons would not be practically or economically feasible for application by the industry.

Table 6. Results of Webb et al. (2015) for in field injections of 200µl of 7.5% levulinic acid (LVA) with 1.0% sodium dodecyl sulfate (SDS) to rockmelons followed by spraying 30ml 7.5% LVA with 0.5% SDS over the entire melon surface immediately at harvest to minmise contamination through transport to the packing shed.

	Log reductions in *L. monocytogenes*			
	Injected and sprayed in the field [a]		Not injected or sprayed in the field [b]	
	Stem scar	Rind	Stem scar	Rind
Chlorine 200ppm	1.4	4.6	0.9	0.8
5%LA + 2%SDS	2.8	5.8	2.4	2.4

[a] Control initial level stem scar 6.7 and rind 7.2 log CFU/sample (3.13cm^3), reductions are following dump tank simulations of 10min

[b] Control initial levels not injected stem scar 6.5 and rind 4.4 log CFU/sample (2.5cm^2), reductions are following dump tank simulations of 8min

8.1.2 Choice of cultivar

The choice of cultivar by farms relies on many factors, but there is limited research to assess whether the level of risk of *L. monocytogenes* differs by cultivars or aspects of different cultivars, such as the degree of netting (*see also* Section 9.2). Nyarko et al. (2016) assessed the effects of cultivar type, storage temperature, and site of contamination on the growth of *L. monocytogenes* on whole rockmelons. 'Athena' and 'Rocky Ford' (associated with the 2011 USA outbreak) varieties were grown from seed in fields and harvested at half or full slip then stored at 4˚C. Melons were brought to ambient temperature and 11cm^2 rind or stem scar sections were inoculated with a three-strain cocktail of *L. monocytogenes* and dried for 1h. Melons were stored at 4, 10 and 25˚C for 15 days. A factorial ANOVA assessed the effects that cultivar, site of inoculation and temperature had on the growth of *L. monocytogenes* on melons. The type of cultivar did not affect the number of *L. monocytogenes* on the surface of whole melons during storage. However, temperature and site of inoculation did, with the number of *L. monocytogenes* decreasing on rind samples across all three test temperatures. In contrast, *L. monocytogenes* proliferated on stem scar sections across all three temperatures. These results suggest that the both the site of

contamination and temperature influence the risk of the proliferation of *L. monocytogenes* under the tested conditions but that these results did not differ by cultivar.

The regular use of sprays and injections in the field to specifically reduce *L. monocytogenes* is unlikely to be feasible for application by the industry. Furthermore, as the prevalence of *L. monocytogenes* in the field is reported to generally be very low, additional application of antimicrobials is not recommended.

We recommend the continued development of science-based risk management information regarding harvest after weather events, including further investigating how long *Listeria* spp. can survive both in soil and on whole melons in the field under different weather conditions in consultation with farmers/producers.

It would be interesting to quantify surface roughness of specific cultivars and investigate whether this influences the potential for *L. monocytogenes* prevalence and growth. Moreover, the influence of cultivar/degree of netting on the efficacy of sanitisation is not well documented. Identification and adoption of low risk cultivars may be a useful additional risk mitigation opportunity for the industry.

8.2 Post-harvest sanitisers and other interventions for reducing the risk of *L. monocytogenes* on melons

As there are no means to completely eliminate *L. monocytogenes* from rockmelons in the field, interventions are also required during processing to reduce the risk from *L. monocytogenes*. There is a desire in the industry to identify interventions able to reliably achieve > 3 $\log_{10}$CFU reductions of *L. monocytogenes* on rockmelons. However, it is important to understand that the choice of any intervention needs to be assessed with consideration of the level of food safety risk reduction both to consumers and the industry against economic, legal, and fruit quality reasons that restrict the sanitiser concentration and contact times applied by the industry. For these reasons, currently used sanitisers are generally applied at manufacturer recommended concentrations for less than 2 min.

This project was *not* intended to provide detailed cost/benefit analysis of rockmelon intervention options: we present here only the results of current research and indicate potentially more effective options the industry may wish to consider. However, we also provide some commentary on relative costs/feasibility of different technologies.

The word 'sanitiser' is used in this report as a term that includes different disinfection interventions including physical, chemical, and biological techniques. All sanitisers in the following review and discussion were applied at temperatures between 20 – 25˚C[10] unless otherwise noted.

NSW DPI (2019b) provides detailed best practice advice regarding the application of sanitisers currently used in Australia. We have provided a brief discussion of some of the factors affecting the efficacy of sanitisers, including contact time and temperature, in Appendix 3.

The results of this review are summarised in three sections:

8.2.1 Currently used post-harvest sanitisation methods for the reduction of *L. monocytogenes* on the surface of melons in Australia;

8.2.2 Potential post-harvest sanitisation methods for the reduction of *L. monocytogenes* on the surface of melons, and;

8.2.3 Other post-harvest interventions for the reduction of *L. monocytogenes* on the surface of melons

The results in Section 8.2.1 and 8.2.2 are presented according to the sanitiser type. In each Section "Introductory" tables (e.g., Sect. 8.2.1.1) are presented that summarise: how the sanitiser works, important considerations for use, and advantages and disadvantages of the sanitiser, as well as "Summary" Tables. The Introductory tables provide references to literature that were not part of the systematic review (because they do not provide information specifically for *L. monocytogenes* and melons) but are included to provide access to further information for readers who are interested in a particular intervention. Introductory tables provide an overview only and are not comprehensive reviews.

Summary tables (e.g., Table 7) present the results from the papers reviewed in detail and provide information regarding the organism, the attachment time (time the bacterial inoculum was given to attach to the rind), the initial $\log_{10}$CFU numbers (number of bacteria attached to the rind before treatment estimated from inoculated untreated rind/melons), the type of sanitiser, the

10 At lower temperature sanitisation takes longer. At 1 to 5°C, the required contact time for a given sanitiser concentration might be 5 – 10 times longer than at 25°C (CDC, 2020; Technical University of Delft, 2020).

concentration/temperature/dose of the sanitiser, the contact time the sanitiser was applied for, and the log_{10}CFU reductions reported for the treatment.

8.2.1 Currently used post-harvest sanitisation methods for the reduction of *L. monocytogenes* on the surface of melons in Australia

The literature search identified seven papers that assessed the efficacy of different sanitisers currently used in Australia to reduce *L. monocytogenes* on the surface of whole melons.

8.2.1.1 Chlorine (hypochlorite)

Chlorine		
What is the status as a fruit/vegetable sanitiser?	Chlorine has been used widely as a water and produce sanitiser, including for rockmelons.	(Ramos et al., 2013; Parish et al., 2003)
What is it?	Chlorine is a highly reactive element that is normally found in nature bound to other elements such as sodium or calcium, however by itself it exists as a gas (Cl_2) that, in high enough concentrations, can be toxic. Chlorine used in liquid sanitisation is generally added in the form of sodium hypochlorite or 'liquid bleach', or powdered forms such as calcium hypochlorite.	(PubChem, 2020a; PubChem, 2020c)
How is it applied?	As a wash or spray	
How does it work?	Hypochlorous acid is the oxidizing agent required for disinfection and transfers across and damages bacterial cell membranes	(McDonnell and Russell, 1999)
How effective is it?	Chlorine is reported to have limited efficacy regardless of concentrations or contact time, and generally reduces microbial loads by < 2 log_{10}CFU on a variety of produce	(Goodburn and Wallace, 2013)
What factors influence effectiveness?	The amount of hypochlorous acid and its effectiveness to inactivate bacteria depends heavily on the pH (required to be between pH 6 – 7.5), the organic load (higher loads reduce efficacy by competing for the sanitiser), and temperature of the water (lower temperatures reduce efficacy). Water flow rate can influence correct auto-dosing.	(Suslow, 1997)
Is it approved or regulated?	Chlorine is currently permitted for use as a processing aid in the *Australia New Zealand Food Standards Code* (the Code): It is a permitted bleaching, washing and peeling agent for all foods: 1mg/kg available chlorine is the maximum permitted level. Commercially available products are currently registered with the APVMA.	(FSANZ, 2016a)
Relative cost?	Low relative cost	
What plant or process changes might be required?	Easily incorporated into current operations	

Are there environmental considerations?	Streams, rivers, and waterways should not be contaminated with these chemicals or used empty containers. Chlorine is carried in the air, where it rapidly reacts to form other compounds. In water, it also reacts rapidly leading to a variety of organochlorine compounds.	(Australian Government, 2018a)
Are there OH&S considerations?	Appropriate handling and storage of concentrates required. Safe Work Australia exposure standards are documented. Appropriate dosing and control to avoid over production of dangerous chlorine gas is required.	(Australian Government, 2018a)
Other Advantages	Readily available. Long history of use.	(Ramos et al., 2013; Parish et al., 2003)
Other Disadvantages	Potential adverse health effects of chlorinated by-products (chloramines). Corrosive to equipment. pH control required at higher concentrations	(Ramos et al., 2013; Parish et al., 2003)
Guidance for use provided to the melon industry	Guidance for the optimised use of chlorine during rockmelon production has previously been provided to the melon industry.	(Suslow, 1997; NSW DPI, 2019a; NSW DPI, 2019b)

Our review identified six papers that specifically assessed the efficacy of chlorine (hypochlorite) to reduce levels of *L. monocytogenes* and other pathogens on melon surfaces. The results are summarised in Table 7.

Ukuku and Fett (2002) assessed 1000ppm chlorine and 5% hydrogen peroxide treatment of whole rockmelons, inoculated by submerging melons in a four-strain cocktail of *L. monocytogenes*. The inoculated melons were stored at 4˚C for up to 15 days before chlorine treatment. After storage, melons were treated by immersion in 1000ppm chlorine (free chlorine was determined with a chlorine test kit) at pH 6.4 (adjusted with citric acid) for 2min. When compared to untreated melons, following storage for 24h, the chlorine treatment significantly reduced *L. monocytogenes* by > 3.2 $\log_{10}$CFU to below the limit of detection (LOD) (LOD 0.3 log CFU/cm^2), and also significantly reduced populations of yeasts and moulds but not below the LOD. In comparison, washing with water alone after 24h storage did not significantly reduce *L. monocytogenes*, native microflora, or yeasts and moulds on surfaces, and only reduced *L. monocytogenes* populations by 0.5 $\log_{10}$ CFU/cm^2. It is also notable that there was no decrease in the effectiveness of water, chlorine (1000ppm) or hydrogen peroxide (5%) treatment of melons inoculated and then stored at 4˚C for 24h, 5 days, or 15 days before treatment. In this study, however, the chlorine concentration was 1000ppm and is not representative of the far lower concentrations (typically 100 - 200 ppm) that are used by the industry and, as the melons were purchased at retail, are not representative of melons coming into a packhouse from the field.

Rodgers et al. (2004) studied the effect of a number of sanitisers, including chlorine at 100 and 200ppm, added to water in the form of chlorinated trisodium phosphate, on *E. coli* O17:H7 and *L. monocytogenes*. Whole rockmelons were stored at 4˚C overnight then inoculated by immersion in three-strain cocktails of either *E. coli* O157:H7 or *L. monocytogenes* for 20min. The rockmelons were then stored for 18 – 24h at 24˚C to allow sufficient time for attachment of the bacteria. The melons were treated by immersion in 100 or 200ppm chlorine (total residual chlorine measured with a colormetric test kit, pH adjusted to 7.0 with lactic acid) for up to 5min with samples taken every 15s and linear $\log_{10}$CFU reduction times determined for each pathogen and sanitiser. The results indicated that washing with sterile water for 5min produced ~1 $\log_{10}$CFU reduction in both pathogens. Treatment with 100ppm and 200ppm chlorine indicated a 1 $\log_{10}$CFU reduction time (*i.e.*, D-value) of 1.01min and 0.92min respectively for *L. monocytogenes*, and similarly, 0.98min and 0.85min for *E. coli*. In that study, both 100pppm and 200ppm chlorine required more time to reduce populations of both *E. coli* and *L. monocytogenes* than 3ppm ozone and both 3 and 5ppm chlorine dioxide (*see* Section 8.2.1.2) but was more effective than 80ppm peracetic acid (*see* Section 8.2.1.3) (which required 1.42min to produce a 1 $\log_{10}$CFU reduction) for the same contact times. However, peracetic acid and chlorine were more effective at reducing yeast and mould populations throughout storage at 4˚C over 9 days. *E. coli* and *L. monocytogenes* populations on treated and untreated melons remained relatively stable throughout storage at 4˚C over 9 days. These results are relevant to the industry as they report more commonly used concentrations and contact times for chlorine (hypochlorite). However, from these data $\log_{10}$CFU reductions of ≤ 2.3 are predicted after 2min exposure to either 100ppm or 200ppm chlorine for both pathogens. Limitations of this study include a lack of data indicating how many samples were taken to estimate the initial numbers of pathogens on the rockmelons and no information on the R^2 of the linear regressions fitted to the data to estimate the subsequent log reductions.

Webb et al. (2015) assessed the efficacy of sanitisers including chlorine, applied in single or double hurdle applications, in reducing *L. monocytogenes*. Fresh harvested rockmelons were obtained from growers prior to washing or packing. For single hurdle applications, rind sections on whole melons were spot inoculated with a five-strain *L. monocytogenes* cocktail, and were treated by submerging the inoculated rockmelons in 10L of either 200ppm chlorine (pH 7.0 adjusted with sulfuric acid, free chlorine was determined with a digital titrator), 3ppm chlorine dioxide or 5% LVA/2.0% SDS for 8min or 10min. The authors reported that *L. monocytogenes* exposed to a single

hurdle application where melons were floated in a dump tank of 200ppm chlorine for 10min were reduced to below the limit of detection (not specified but we estimated a log_{10}CFU reduction of >2.7) on the rind and 1.1 log_{10}CFU on the stem scar. When melons were floated in a dump tank of 200ppm chlorine for 8min, *L. monocytogenes* was reduced by 0.8 log_{10}CFU on the rind and 0.9 log_{10}CFU on the stem scar. A double hurdle application of 200 ppm chlorine in a dump tank (10min) followed by 5% levulinic acid/2.5% SDS in a dip tank (1min), produced a 1.4 log_{10}CFU reduction at the stem scar and reductions below the LOD on the rind, and were no more effective than the single hurdle applications of chlorine for 10min. No explanation for the discrepancy in the rind log_{10}CFU reductions of the single hurdle applications was provided by the authors. Regardless, these contact times are not tenable under current practices in the Australian industry, and particularly because dump tanks are no longer used.

Upadhyay et al. (2016) assessed the efficacy of 200ppm chlorine and octenidine dihydrochloride washes (*see* Section 8.2.2.6) and coatings (Section 8.2.2.7) to reduce *L. monocytogenes*, *E. coli* and *Salmonella* on rockmelon surfaces. Whole melons purchased at retail were inoculated by immersion in 3L of a four-strain cocktail of either *L. monocytogenes*, *E. coli* or *Salmonella* and dried for 2h. Whole melons were then immersed in 3L of 200ppm total chlorine (pH not reported) for 5min at 25˚C. Rind plug sections were removed and enumerated. 200ppm chlorine applied for 5min achieved ≤ 1.1 log_{10}CFU kill for all pathogens and was less effective than octenidine dihydrochloride (*see* Section 8.2.2.6).

Svoboda et al. (2016) intended to determine the effectiveness of a number of sanitisers, including 200ppm chlorine (in the form of commercial bleach) against *E. coli*, *Listeria innocua*[11] and *Salmonella* on the surface of whole rockmelons and watermelons. Previously untreated melons (3 days from harvest) were purchased and first washed with tap water and stored at room temperature. The melons were inoculated by immersion in three-strain cocktails of either *E. coli* O157:H7, *E. coli* STEC, *L. innocua* or *Salmonella*. The melons were sanitised by immersion in 5L of 200ppm free chlorine (pH not reported) for 2min with manual agitation, then rinsed with sterile tap water for 5s. Those authors reported no significant differences in the reductions for any pathogen on rockmelons or watermelons and therefore chose to combine the results from each

11 *L. innocua* is virtually genetically identical to *L. monocytogenes* except that it lacks the key virulence genes of *L. monocytogenes*. In all other respects it behaves nearly identically to *L. monocytogenes* and is widely used as a 'safe' alternative for experimental studies that consider the ecophysiology of *L. monocytogenes* but that do not involve studies of its virulence or pathogenicity.

melon type for each sanitiser. However, the statistical analyses in that paper are not appropriate (A. Gracie, pers. comm., 2020), and their decision to pool data that was presented in the accompanying thesis (Svoboda, 2015) are not justified and distort the findings presented and the reliability of their interpretation. Therefore, the results of the published paper are not an accurate, nor reliable, analysis of the effects of the sanitisers on the tested pathogens for either watermelon or rockmelon. The log_{10}CFU reductions for 2min exposure to 200ppm chlorine were 0.6 for *E. coli* O157:H7, 0.6 for *E. coli* STEC, 1.9 for *L innocua*, and 1.2 for *Salmonella.* The reported results for *L. innocua* and *Salmonella* are similar to those reported earlier (Rodgers et al., 2004). Moreover, when compared against nine other sanitisers or simply water, chlorine did not cause significantly greater reductions of *L. monocytogenes* than any other sanitiser.

Singh et al. (2018) assessed the effectiveness of a number of sanitisers, including 100ppm chlorine, against *L. monocytogenes* and *Salmonella* on the surface of whole rockmelons. Rockmelons were purchased at retail and 16cm^2 rind sections on whole melons were marked and spot inoculated with five-strain cocktails of either *L. monocytogenes* or *S*. Typhimurium. Whole melons were immersed for 5min in 15L of 100ppm free chlorine (pH not reported, free chlorine was determined with a digital titrator) chilled at 4˚C. The choice of 4˚C for the temperature of the sanitiser treatment used in this study is an important departure from the previous studies described earlier, all of which tested sanitisers at temperatures from 20˚C – 25˚C: no reason for this choice of temperature was stated. Treatment with 100ppm chlorine for 5min produced log_{10}CFU reductions of 1.9 and 3.8 for *L. monocytogenes* and *S.* Typhimurium respectively. However, 100ppm chlorine was less effective at reducing populations of *L. monocytogenes* than peracetic acid (45, 85, 100ppm) (*see* Section 8.2.1.3), electrolysed water (100ppm)(*see* Section 8.2.2.13), or 2% lactic acid (*see* Section 8.2.2.5) also tested in that study. For *S*. Typhimurium, only peracetic acid (85, 100ppm) was more effective than 100ppm chlorine. While the study tested commercially relevant concentrations of chlorine, the application of the sanitiser was at 4˚C. It is known that the effectiveness of chlorine is lessened at lower temperature (Suslow, 1997). Moreover, the contact time (5min) used in this study is longer than that used by most Australian packhouses to achieve their daily production throughput. It should also be noted that this study was, in part, funded by the manufacturer of the peracetic acid used in the study.

Six authoritative studies were found that provided detailed evaluation of the efficacy of chlorine sanitisers to reduce *L. monocytogenes* and other pathogens on the surface of whole melons.

Log_{10}CFU reductions of > 3 for *L. monocytogenes* on the surface of rockmelons were not achieved in any study that assessed chlorine at 100 or 200ppm for a 2 minute contact time. In studies that compared a number of sanitisers, chlorine-based sanitiser were never the most effective option at industry relevant contact times.

There are limited published, authoritative, studies concerning chlorine-based sanitisers, at industry-relevant contact times and concentrations. The few studies available indicate that 100ppm or 200pm concentrations for 2min at ambient temperature can achieve a ~2 log reduction in *Listeria on* the surface of whole melons.

Table 7. Studies that have assessed the efficacy of chlorine (hypochlorite) sanitisers to reduce *L. monocytogenes* and other pathogens on the surface of rockmelons.

Reference	Organism	Attachment time	Initial Log_{10}CFU numbers	Sanitiser	Concentration (ppm)	Contact time (min)	Log_{10}CFU reduction
Ukuku and Fett (2002)	*L. monocytogenes*	24h	3.5	Chlorine	1000	2.0	> 3.2
Ukuku and Fett (2002)	*L. monocytogenes*	24h	3.5	Water		2.0	0.5
Rodgers et al. (2004)	*L. monocytogenes*	24h	~6.0	Chlorine	100	2.0 to 5.0	*2.0 to >5
Rodgers et al. (2004)	*L. monocytogenes*	24h	~6.0	Chlorine	200	2.0 to 5.0	*2.2 to >5
Rodgers et al. (2004)	*E. coli*	24h	~6.0	Chlorine	100	2.0 to 5.0	*2.0 to >5
Rodgers et al. (2004)	*E. coli*	24h	~6.0	Chlorine	200	2.0 to 5.0	*2.3 to >5
Webb et al. (2015)	*L. monocytogenes*	18h	4.0	Chlorine	200	10.0	> 2.7#
Webb et al. (2015)	*L. monocytogenes*	18h	4.0	Chlorine	200	8.0	0.8
Upadhyay et al. (2016)	*Salmonella*	2h	5.6	Chlorine	200	5.0	0.5
Upadhyay et al. (2016)	*L. monocytogenes*	2h	5.6	Chlorine	200	5.0	0.9
Upadhyay et al. (2016)	*E. coli*	2h	5.6	Chlorine	200	5.0	1.1

Svoboda et al. (2016)	*E. coli* O157:H7	1h	4.2	Chlorine	200	2.0	0.6
Svoboda et al. (2016)	*E. coli* STEC	1h	3.3	Chlorine	200	2.0	0.6
Svoboda et al. (2016)	*L innocua*	1h	4.5	Chlorine	200	2.0	1.9
Svoboda et al. (2016)	*Salmonella*	1h	3.8	Chlorine	200	2.0	1.2
Singh et al. (2018)	*L. monocytogenes*	overnight	7.6	Chlorine	200	5.0	1.9
Singh et al. (2018)	*S. Typhimurium*	overnight	6.6	Chlorine	200	5.0	3.8

* indicates $\log_{10}$CFU reductions for 2 min contact times calculated from linear log reduction times reported by Rodgers et al. (2004) to allow for ease of comparison between studies. At 5 min, the maximum treatment duration, all counts were below the limit of detection.
> indicates results below the limit of detection
# indicates limit of detection not reported, estimate based on a 1.3 $\log_{10}$CFU LOD.

8.2.1.2 Chlorine dioxide (aqueous)

Chlorine dioxide (aqueous)		
What is the status as a fruit/vegetable sanitiser?	Chlorine dioxide is currently commercially available as an aqueous-based sanitiser for fresh produce.	(Praeger et al., 2018)
What is it?	Chlorine dioxide (ClO_2) is a small molecule that is highly soluble in water and can be generated in solution by adding hydrochloric acid and sodium chlorite together. 'Aqueous chlorine dioxide' refers to chlorine dioxide dissolved in water.	(Praeger et al., 2018)
How is it applied?	Aqueous chlorine dioxide is applied as a wash or spray to produce.	
How does it work?	The antimicrobial activity is mainly attributed to damage to the cell membrane but other mechanisms have been discussed.	(Praeger et al., 2018)
How effective is it?	Chlorine dioxide is reported to be effective in reducing bacterial, viral and fungal contaminants. When generated in water, chlorine dioxide does not dissociate but exists as a free radical and is reported to have 2.5 times more oxidation capacity than chlorine at equivalent concentrations	(Benarde et al., 1965)
What factors influence effectiveness?	Concentration, contact time, produce type, temperature. Not affected by organic load. Less affected by pH than chlorine.	(Parish et al., 2003; Ramos et al., 2013)
Is it approved or regulated?	Chlorine dioxide is currently permitted for use as a processing aid in the *Australia New Zealand Food Standards Code* (the Code): Permitted for bleaching, washing and peeling agents, all foods, 1mg/kg available chlorine maximum permitted level. Commercial products are currently registered with the APVMA.	(FSANZ, 2016a)
Relative cost?	Relatively low cost, but more expensive than chlorine at recommended concentrations.	
What plant or process changes might be required?	The technology to use this sanitiser is readily commercially available: the sanitiser is generated in-house using commercially available components and automated dosing machines. Can be incorporated into existing sanitation processes.	
Are there environmental considerations?	Streams, rivers, and waterways should not be contaminated with these chemicals or used empty containers. Because of its high reactivity, chlorine dioxide will not persist long in air, water, or soil environments – it will survive for up to minutes in air and up to hours in water or soils.	(Australian Government, 2018b)
Are there OH&S considerations?	Safe handling and storage of chemicals is required for onsite generation. Safe Work Australia exposure standards are documented.	(Australian Government, 2018b)
Other Advantages	Less reactivity than hypochlorite with organic compounds. Fewer chlorinated by-products are created. Better antimicrobial activity at neutral pH than hypochlorites. Not corrosive at permitted levels.	(Parish et al., 2003; Ramos et al., 2013)
Other Disadvantages	Requires on-site generation. Potentially explosive. More iodinated DBP formation than chlorine if iodide is present in water. Formation of specific by-products, chlorite and chlorate.	(Parish et al., 2003; Ramos et al., 2013)

Guidance for use provided to the melon industry	Guidance for the optimised use of chlorine dioxide during rockmelon production has previously been provided to the melon industry	(NSW DPI, 2019a; NSW DPI, 2019b)

Three relevant papers were identified that assessed the efficacy of aqueous chlorine dioxide against *L. monocytogenes* and other pathogens on the surface of rockmelons. The results of the relevant studies are presented in Table 8.

Rodgers et al. (2004), discussed in Section 8.2.1.1, studied the efficacy of a number of sanitisers, including 3 and 5ppm chlorine dioxide in solution to inactivate *E. coli* O17:H7 and *L. monocytogenes* on whole rockmelons. The same inoculation methods were used as for chlorine. The inoculated melons were immersed in 3 or 5ppm chlorine dioxide for up to 5min with samples taken every 15s to allow for the determination of $\log_{10}$CFU reduction times for each sanitiser. 3 and 5 ppm chlorine dioxide required 0.83 and 0.69min respectively to achieve a 1 $\log_{10}$CFU reduction of *L. monocytogenes*, and 0.74 and 0.71min for a 1 $\log_{10}$CFU reduction for *E. coli.* In that study chlorine dioxide (5ppm) and ozone (3ppm) were the most effective sanitisers compared to peracetic acid (85ppm) and chlorine (100ppm). While chlorine dioxide (5ppm) and ozone (3ppm) were the most effective against *L. monocytogenes* and *E. coli,* they were the least effective at suppressing mould and yeast throughout storage. This study demonstrated that at an industry relevant contact time of 2min, 3ppm and 5ppm chlorine dioxide produced $\log_{10}$CFU reductions of 2.4 and 2.9 in *L. monocytogenes* on the surface of melons.

Webb et al. (2015) assessed the efficacy of sanitisers, including chlorine dioxide, to reduce *L. monocytogenes* applied in single or double hurdle applications. Freshly harvested rockmelons were obtained from growers prior to washing or packing. The same inoculation methods as for chlorine treatment were used (Section 8.2.1.1) and melons were then treated with 3ppm chlorine dioxide for 8min. Webb et al. (2015) reported that there was no reduction of *L. monocytogenes* inoculated on the rind, and a 0.2 $\log_{10}$CFU reduction at the stem scar, and this is an unusual result given the long contact time of 8min. As for chlorine, these contact times are not tenable under current practices in the Australian industry.

Svoboda et al. (2016), introduced in Section 8.2.1.1, aimed to determine the effectiveness of a number of sanitisers, including 5ppm chlorine dioxide in solution against strains of *E. coli*, *L.*

innocua and *Salmonella* on the surface of whole rockmelons and watermelons. The same inoculation methods were used as for chlorine, and inoculated melons were immersed in 5ppm chlorine dioxide for 2min. The log_{10}CFU reductions were 1.6 for *E. coli* O157:H7, 1.6 for *E. coli* STEC, 0.8 for *L innocua*, and 2.1 for *Salmonella.* In the study, for all cases except *L. innocua,* 5ppm chlorine dioxide for 2min was more effective than 100ppm chlorine for the same contact time, however, it was not the most effective sanitiser among those tested for any pathogen in that study. Importantly, as discussed in Section 8.2.1.1, the reliability of these results and their interpretation are questionable.

To summarise, the results of Rodgers et al. (2004) showed consistently larger reductions for all pathogens for both 3ppm and 5ppm chlorine dioxide with a contact time of 2min compared to the results of Svoboda et al. (2016) for 5ppm. Although the results suggest aqueous chlorine dioxide was generally more effective than chlorine (hypochlorite), a contact time of 2min is still required.

Three relevant papers were identified that assessed the efficacy of aqueous chlorine dioxide against *L. monocytogenes* and other pathogens on the surface of rockmelons.

Log_{10}CFU reductions of > 3 for *L. monocytogenes* on the surface of rockmelons were not achieved in any study that assessed aqueous chlorine dioxide at 3ppm or 5ppm for 2min contact time treatments.

There are limited studies at industry-relevant contact times and concentrations. The limited number of studies reported log reductions of 0.8 - 2.9 of *Listeria* on the surface of melons for 3ppm or 5ppm chlorine dioxide (aqueous) applied for 2min.

Table 8. Studies assessing chlorine dioxide applied in water to reduce the population of *L. monocytogenes* and other pathogens from rockmelon surfaces.

Reference	Organism	Attachment time	Initial Log_{10}CFU numbers	Sanitiser	Concentration (ppm)	Contact time (min)	Log_{10}CFU reduction
Rodgers et al. (2004)	*L. monocytogenes*	24h	~6.0	Chlorine dioxide	3	2.0 to 5.0	*2.4 to >5
Rodgers et al. (2004)	*L. monocytogenes*	24h	~6.0	Chlorine dioxide	5	2.0 to 5.0	*2.9 to >5
Rodgers et al. (2004)	*E. coli*	24h	~6.0	Chlorine dioxide	3	2.0 to 5.0	*2.7 to >5
Rodgers et al. (2004)	*E. coli*	24h	~6.0	Chlorine dioxide	5	2.0 to 5.0	*2.8 to >5
Webb et al. (2015)	*L. monocytogenes*	18h	4.4	Chlorine dioxide	3	8.0	-0.8
Svoboda et al. (2016)	*E. coli* O157:H7	1h	4.2	Chlorine dioxide	5	2.0	1.6
Svoboda et al. (2016)	*E. coli* STEC	1h	3.3	Chlorine dioxide	5	2.0	1.6
Svoboda et al. (2016)	*L innocua*	1h	4.5	Chlorine dioxide	5	2.0	0.8
Svoboda et al. (2016)	*Salmonella*	1h	3.8	Chlorine dioxide	5	2.0	2.1

* indicates log_{10}CFU reductions for 2 min contact times calculated from linear log reduction times reported by Rodgers et al. (2004) to allow for ease of comparison between studies. At 5 min, the maximum treatment duration,, all counts were below the limit of detection.

8.2.1.3 Peracetic (or peroxyacetic) acid

Peracetic acid		
What is the status as a fruit/vegetable sanitiser?	Peroxyacetic acid is a commercially available vegetable and fruit sanitiser generally used at 80ppm. Also used in water treatment and health care.	(Ramos et al., 2013)
What is it?	Peroxyacetic acid (CH_3COOOH) is an organic compound that is a strong oxidising agent. Solutions of peracetic acid used as sanitisers are formed by the combination of acetic acid and hydrogen peroxide. Acetic acid and hydrogen peroxide react to form an equilibrium solution containing peracetic acid, acetic acid and hydrogen peroxide that is sold commercially as the sanitiser "peracetic acid."	(PubChem, 2020g)
How is it applied?	As a wash or spray.	
How does it work?	Peroxyacetic acid denatures proteins and lipids in the bacterial cell membrane.	(Wessels and Ingmer, 2013)
How effective is it?	Broadly bactericidal. Low antimicrobial efficacy at permitted levels for vegetables.	(Ramos et al., 2013)
What factors influence effectiveness?	The food matrix. Contact time. Not affected by water organic load. Not affected by temperature changes and can be used at low temperatures. Effective over a wide pH range 5 - 8	(Ramos et al., 2013)
Is it approved or regulated?	Peroxyacetic acid is currently permitted for use as a processing aid in the *Australia New Zealand Food Standards Code* (the Code). Permitted bleaching, washing and peeling agent, all foods, GMP maximum permitted level. Commercial products are currently registered with the APVMA.	(FSANZ, 2016a)
Relative cost?	More expensive than chlorine at recommended dose levels.	
What plant or process changes might be required?	Can be easily incorporated into current systems.	
Are there environmental considerations?	Streams, rivers, and waterways should not be contaminated with these chemicals or used empty containers.	
Are there OH&S considerations?	Safe handling and storage of chemicals. Safe Work Australia exposure standards are being reviewed	(Safe Work Australia, 2020)
Other Advantages	No harmful disinfection by-products formed. Not corrosive at permitted levels (80 ppm).	(Ramos et al., 2013)
Other Disadvantages	Reacts with chlorine to form dangerous chlorine gas. Not all commercially available peroxyacetic acid is the same formula.	(Ramos et al., 2013)
Guidance for use provided to the melon industry	Guidance for the optimised efficacy of peroxyacetic acid during rockmelon production has previously been provided to the melon industry	(NSW DPI, 2019a; NSW DPI, 2019b)

The review identified four papers that investigated the ability of peracetic acid (PAA) to reduce the number of *L. monocytogenes* and other pathogens on the surface of melons. The results are summarised in Table 9.

Rodgers et al. (2004), introduced in Section 8.2.1.1, studied the effect of a number of sanitisers, including 80ppm peracetic acid, on reducing *E. coli* O17:H7 and *L. monocytogenes* on whole rockmelons. The same inoculation methods as for chlorine treatment were used (described in Section 8.2.1.1). The inoculated melons were immersed in 80ppm PAA for up to 5min with samples taken every 15s to determine linear $\log_{10}$CFU reduction times for each sanitiser. 80ppm PAA required 1.42min to achieve a 1 $\log_{10}$CFU reduction of both *L. monocytogenes*, and *E. coli,* and compared to all other sanitisers tested (100 and 200ppm chlorine, 3 and 5ppm chlorine dioxide, 3ppm ozone) was the *least* effective for reducing numbers of both organisms.

Suslow and Callejas (2015) assessed the efficacy of hot water (63-68˚C) applied via a laboratory scale thermal shower with and without a subsequent pressure spray application of 30ppm PAA, but here we discuss the results for PAA only. Field-packed rockmelons were obtained from a wholesaler and stored at 2.5˚C before use within two days. Whole melons were spot inoculated on two 5cm diameter circles with either an attenuated *Salmonella* Typhimurium or *L. innocua* inoculum. The melons were dried for 4h at 22˚C then transferred to 2.5˚C for 3 days before treatment. Melons were then pressure sprayed for 1 sec with 30 ppm peroxyacetic acid or not washed, for comparison. The results demonstrated that 30ppm PAA applied for 1 sec reduced *S.* Typhimurium and *L. innocua* by 2.1 and 1.9 $\log_{10}$CFU respectively. The action of the pressure spray may have contributed to the reductions that are quite large for such a small contact time. This highlights the need to understand the effect that different applications of sanitisers (e.g. spray versus immersion) may have on pathogen reductions. These $\log_{10}$CFU reductions are the least effective of the methods tested in Suslow and Callejas (2015) (*see* Section 8.2.2.3).

Svoboda et al. (2016), introduced in Section 8.2.1.1, also assessed different sanitisers. However, as noted earlier, due to their approaches to data analysis, their results may be unreliable[12]. The maximum $\log_{10}$CFU reduction in 2min was not greater than 2.6 $\log_{10}$CFU for any pathogen and any brand of PAA tested in this study.

12 Due to the unreliability of the results provided in Svoboda et al. (2016) we have not reported their results for other potential sanitisers not currently used by the Australian industry.

Singh et al. (2018) reported on the effectiveness of a number of sanitisers, including 45, 85, and 100ppm peracetic acid, against *L. monocytogenes* and *Salmonella* on the surface of whole rockmelons. In that study, washing melons in 100ppm peracetic acid (at 4˚C) for 5min was the most effective sanitiser against the two pathogens (> 4 log_{10}CFU reductions). 85ppm for 5min produced 3 and 4.2 log_{10}CFU reductions of *L. monocytogenes* and *S.* Typhimurium respectively. However, 100ppm and 5min are concentrations and times that are not currently used within the Australian industry and it is unlikely this combination would be feasible within the industry. Moreover, this study was undertaken with all sanitisers at 4˚C which is not representative of melon industry conditions. It is known that lower temperatures reduce the efficacy of some sanitisers (Suslow, 1997) and could explain the limited effectiveness of the other sanitisers tested in the study.

Four studies have investigated the ability of peracetic acid (PAA) to reduce the number of *L. monocytogenes* and other pathogens on the surface of melons.

Log_{10}CFU reductions of > 3 for *L. monocytogenes* on the surface of rockmelons were not achieved in any study that assessed PAA at 30ppm to 100ppm for 2min contact times.

There are limited studies at industry-relevant contact times and concentrations. The limited number of studies reported log reductions of 1.4 - 2.1 of *L. monocytogenes* on the surface of melons for 30ppm to 100ppm PAA when applied for 2min.

Even though a 5min contact time for 100ppm PAA produced high reductions (4.5 Log) these conditions are not feasible in the Australian industry.

Table 9. Studies assessing peracetic acid (PAA) to reduce populations of *L. monocytogenes* and other pathogens on rockmelon surfaces.

Reference	Organism	Attachment time	Initial Log_{10}CFU numbers	Sanitiser	Concentration (ppm)	Contact time (min)	Log_{10}CFU reduction
Rodgers et al. (2004)	*L. monocytogenes*	24h	6	PAA	80	2.0 to 5.0	*1.4 to >5
Rodgers et al. (2004)	*E. coli*	24h	6	PAA	80	2.0 to 5.0	*1.4 to >5
Suslow and Callejas (2015)	*L. innocua*	76h	3.9	PAA	30	1 second	1.9
Suslow and Callejas (2015)	*Salmonella*	76h	3.5	PAA	30	1 second	2.1
Svoboda et al. (2016)	*E. coli* O157:H7	1h	4.2	PAA (a)	100	2.0	2.3
Svoboda et al. (2016)	*Salmonella*	1h	3.8	PAA (a)	100	2.0	2.0
Svoboda et al. (2016)	*L. innocua*	1h	4.5	PAA (a)	100	2.0	1.8
Svoboda et al. (2016)	*E. coli* non-O157 STEC	1h	3.3	PAA (a)	100	2.0	1.6
Svoboda et al. (2016)	*Salmonella*	1h	3.8	PAA (b)	100	2.0	2.6
Svoboda et al. (2016)	*Listeria innocua*	1h	4.5	PAA (b)	100	2.0	1.5
Svoboda et al. (2016)	*E. coli* non-O157 STEC	1h	3.3	PAA (b)	100	2.0	0.9
Svoboda et al. (2016)	*E. coli* O157:H7	1h	4.2	PAA (b)	100	2.0	0.9
Singh et al. (2018)	*L. monocytogenes*	Overnight	7.6	PAA (c)	100	5.0	4.5
Singh et al. (2018)	*S. Typhimurium*	Overnight	6.6	PAA (c)	100	5.0	4.5
Singh et al. (2018)	*S. Typhimurium*	Overnight	6.6	PAA (c)	85	5.0	4.2
Singh et al. (2018)	*S. Typhimurium*	Overnight	6.6	PAA (c)	45	5.0	3.6
Singh et al. (2018)	*L. monocytogenes*	Overnight	7.6	PAA (c)	85	5.0	3.0
Singh et al. (2018)	*L. monocytogenes*	Overnight	7.6	PAA (c)	45	5.0	3.0

* indicates log_{10}CFU reductions for 2 min contact times calculated from linear log reduction times reported by Rodgers et al. (2004) to allow for ease of comparison between studies. At 5 min, the maximum treatment duration, all counts were below the limit of detection.
(a) SaniDate 12.0
(b) StorOx 2.0
(c) VigorOx 15 F&V

8.2.1.4 Ozone (Aqueous)

Ozone (aqueous)		
What is the status as a fruit/vegetable sanitiser?	Ozone is an antimicrobial agent commonly used for food and agricultural applications including melon sanitisation.	(Ramos et al., 2013; Parish et al., 2003)
What is it?	Ozone (O_3) is a naturally occurring elemental molecule comprising of three oxygen atoms. Ozone is formed by splitting molecular oxygen (O_2) with a high energy input. The oxygen molecules rapidly combine with available O_2 to form O_3. Ozone rapidly degrades to O_2.	(PubChem, 2020f)
How is it applied?	Once ozone has been formed it can be applied as a gas or added to water.	(Miller et al., 2013)
How does it work?	Ozone damages the bacterial cell membrane or penetrates the cell and oxidises vital cell components.	(Miller et al., 2013)
How effective is it?	High antimicrobial activity. Broad spectrum. Good penetration ability. Effectiveness against protozoa reported.	(Ramos et al., 2013; Parish et al., 2003)
What factors influence effectiveness?	Contact time, pressure, temperature, pH, bubble size, flow rate, and water purity. Higher temperatures and pH will decrease ozone stability.	(Miller et al., 2013)
Is it approved or regulated?	Ozone is currently permitted for use as a processing aid in the *Australia New Zealand Food Standards Code*. It is a permitted bleaching, washing and peeling agent, for all foods, GMP maximum permitted level.	(FSANZ, 2016a)
Relative cost?	Higher initial capital investment cost for dosing equipment. Lower running cost	(Ramos et al., 2013; Parish et al., 2003)
What plant or process changes might be required?	Can be incorporated into existing operations.	
Are there environmental considerations?	Due to the instability of ozone it does not produce residues or wastewater issues.	(Ölmez and Kretzschmar, 2009)
Are there OH&S considerations?	Possible human toxic effects in enclosed spaces in processing facilities. Requires monitoring in indoor applications. Safe Work Australia exposure standards are documented.	(Ramos et al., 2013; Parish et al., 2003)
Other Advantages	Generally recognized as safe (GRAS). No hazardous disinfection by-products are formed and does not leave hazardous residues on food.	(Ramos et al., 2013; Parish et al., 2003)
Other Disadvantages	Possible deterioration of produce flavour and colour. Can cause physiological injury and loss of antioxidant constituents in produce. Unstable, very highly reactive. Requires on-site generation. Not compatible with chlorine or bromide. Corrosive to equipment.	(Ramos et al., 2013; Parish et al., 2003)
Guidance for use provided to the melon industry	Guidance for the optimised efficacy of ozone during rockmelon production has previously been provided to the melon industry	(NSW DPI, 2019a; NSW DPI, 2019b)

Our literature search revealed only one study that investigated the effectiveness of aqueous ozone specifically for reduction of *L. monocytogenes* on rockmelon surfaces. Rodgers et al. (2004), first introduced in Section 8.2.1.1, reported that 3ppm ozonated water and 5ppm aqueous chlorine dioxide were the most effective sanitisers tested compared to chlorine dioxide (3 ppm), chlorine (200ppm and 100ppm) and peracetic acid (80ppm). The Log_{10}CFU reductions reported for *L. monocytogenes* and *E. coli* are presented in Table 10 and represent the largest reported Log_{10}CFU reductions for both pathogens compared to the other currently used sanitisers applied for a 2min contact time. Aqueous ozone is also a technology that is currently commercially available to the Australian industry and can be readily incorporated into existing infrastructure. However, more research to assess the efficacy of different concentrations/contact times and the effect on melon quality is required.

There are very few studies at industry-relevant contact times and concentrations for ozone efficacy against *L. monocytogenes* on rockmelons.

A Log_{10}CFU reduction of 3 for *L. monocytogenes* on the surface of rockmelons was reported in a single study (Rodgers *et al.,* 2004) that assessed aqueous ozone at 3ppm applied for 2min.

Given this result, it is perhaps surprising that the efficacy of ozone as a sanitisation treatment for *L. monocytogenes* on rockmelon has not been more often studied and described in the published, authoritative, literature.

Table 10. Summary of the published study that has assessed the efficacy of ozone (aqueous) to reduce *L. monocytogenes* and other pathogens on the surface of rockmelons.

Reference	Organism	Attachment time	Initial Log_{10}CFU numbers	Sanitiser	Concentration (ppm)	Contact time (min)	Log_{10}CFU reduction
Rodgers et al. (2004)	*L. monocytogenes*	18h	6.0	Ozone	3	2.0 to 5.0	*3.0 to >5
Rodgers et al. (2004)	*E. coli*	18h	6.0	Ozone	3	2.0 to 5.0	*2.9 to >5

* indicates log_{10}CFU reductions for 2 min contact times calculated from linear log reduction times reported by Rodgers et al. (2004) to allow for easy of comparison between studies. At 5 min, the end of the experiment, all counts were below the limit of detection.

8.2.1.5 Conclusions regarding currently used post-harvest sanitisation methods for the reduction of *L. monocytogenes* on the surface of melons in Australia

There is little published research concerning sanitisers currently used in the Australian rockmelon industry at industry relevant concentrations and contact times, for the combination of *L. monocytogenes* on rockmelon rind, to provide the industry with confidence in their efficacy. For this reason it is recommended that further research into these sanitisers *relevant to Australian industry conditions* be continued.

We conclude that there is insufficient research to confidently specify recommendations for optimal contact times for sanitisers, both for product quality and safety, specifically for *L. monocytogenes*. Nonetheless, in the absence of more evidence our results support the current recommendations of NSW DPI (2019b) for chlorine (100ppm), peroxyacetic acid (80ppm), and chlorine dioxide (aqueous; 5ppm) for contact times of 2 minutes. Due to the potential limits to efficacy, the overall effectiveness of sanitisers used in Australia will depend on the consistent implementation of a whole-chain approach to rockmelon food safety.

8.2.2 Potential post-harvest sanitisation methods for the reduction of *L. monocytogenes* on the surface of melons

Stakeholders in the Australian melon industry expressed a desire to identify research into other potential sanitisers that may have an increased efficacy in killing or removing *L. monocytogenes* on the surface of whole rockmelons. Below we have summarised the available research for different potential sanitisers/interventions relevant to post-harvest processing that were identified in our review of the published literature.

8.2.2.1 Chlorine dioxide (gaseous, and sequential application of sodium chlorite and hydrochloric acid)

Chlorine dioxide (gaseous sequential application of sodium chlorite and hydrochloric acid)		
What is the status as a fruit/vegetable sanitiser?	Chlorine dioxide gas has received increasing attention as an alternative produce sanitiser, however, due to the explosive nature of the gaseous form of the compound it has had limited commercial application in the fresh produce industry.	
What is it?	Gaseous chlorine dioxide (ClO_2) is a small molecule that can be generated by reacting two substances together. It is difficult to prepare in an industrial setting and cannot be prepared 'off-site' and transported to the processing plant as ready-to-use chemical.	(Praeger et al., 2018)
How is it applied?	Chlorine dioxide gas requires a sealed chamber for the gas to then be pumped in to, and contain the gas, to be able to treat produce. Dipping melons in sodium chlorite and hydrochloric acid for sequential application.	
How does it work?	Chlorine dioxide gas is a strong oxidising agent and attacks and penetrates bacterial cell membranes.	(Sun et al., 2019)
How effective is it?	Reported to be effective against a range of microorganisms.	(Sun et al., 2019)
What factors influence effectiveness?	Effectiveness of chlorine dioxide gas depends on factors such as concentration, contact time, relative humidity, temperature, and the uniformity of gas distribution inside the treatment chamber. Undetermined for sequential application of sodium chlorite and hydrochloric acid	(Ramos et al., 2013)
Is it approved or regulated?	Requires consultation with state authorities, APVMA and/or FSANZ before use regarding requirements for approvals.	
Relative cost?	Considerable initial cost for gas generating equipment. Undetermined for sequential application of sodium chlorite and hydrochloric acid	
What plant or process changes might be required?	Generation of chlorine dioxide gas requires specialist equipment and a sealed chamber. Process changes would be required to incorporate this before or after packaging. Undetermined for sequential application of sodium chlorite and hydrochloric acid	
Are there environmental considerations?	If it occurs, releases will be as chlorine dioxide gas to the atmosphere or in wastewater streams from plants that make or use chlorine dioxide. Because of its high reactivity chlorine dioxide will	(Australian Government, 2018b)

	not persist long in the air, water, or soil environments – up to minutes in air and up to hours in the soil or water.	
Are there OH&S considerations?	Chlorine dioxide gas is flammable, and is highly explosive in air at concentrations > 10%. Safe Work Australia exposure standards are documented. Storage of concentrated chemicals for sequential application of sodium chlorite and hydrochloric acid	(Australian Government, 2018b)
Other Advantages	Potential for increased penetration by gas.	
Other Disadvantages	Gas cannot be transported and must be generated on site.	

Our review identified two papers that reported investigations on the efficacy of chlorine dioxide *gas*, and one paper investigating the sequential application of sodium chlorite and hydrochloric acid to generate chlorine dioxide on rind to reduce *L. monocytogenes* and other pathogens on rockmelon surfaces. The results are presented in Table 11.

Mahmoud et al. (2008) aimed to determine the effect of different concentrations of chlorine dioxide gas on *E. coli*, *S.* Poona, and *L. monocytogenes* and also on the melon quality and shelf life. Rockmelons were purchased at retail and 5cm^2 marked areas on the surface of the melons were spot inoculated with either a three-strain cocktail of *E. coli* O157:H7 or *L. monocytogenes* or a single strain of *S.* Poona. The inoculated melons were placed in a treatment chamber and exposed to 0.5, 1.0, 1.5, 3.0 and 5.0mg/L chlorine dioxide gas for 10min. Chlorine dioxide gas was generated based on the reaction of 4% chlorine gas with sodium chlorite. The Weibull model was used to analyse the inactivation data and to determine $\log_{10}$CFU reduction times. Sensory analysis was also undertaken with uninoculated rockmelons treated and stored at 22˚C for 12 days and colour, yeasts, moulds, mesophilic, and psychrotrophic bacteria were then assessed. In all cases, the survival curves of all pathogens treated with gas were not log-linear and the rate of inactivation of target cells was faster during earlier stages of exposure. Exposure for 2min at the higher concentrations of either 3 or 5mg/l was found to achieve a 2 – 3.2 $\log_{10}$CFU reduction for all pathogens. The estimated time for a 3 $\log_{10}$CFU reduction at 5mg/L was 4.2min for *L. monocytogenes*, 5.5min for *E. coli*, and 1.5min for *Salmonella*. Treatment with chlorine dioxide gas did not affect the external or internal colour of melons but did significantly reduce native microbiota, and treatment with 5 mg/L extended shelf life by six days compared to the (untreated) control stored at 22˚C. The Mahmoud et al. (2008) study produced valuable information regarding exposure time for low concentration treatment with chlorine dioxide gas, however, the time required to achieve > 3 Log_{10}CFU reduction was > 4min.

Trinetta et al. (2013) expanded on the research described above by investigating higher concentrations of chlorine dioxide gas applied for 3min. Whole rockmelons were obtained from commercial distributors and 6.5cm^2 sections were spot inoculated with a three-strain cocktail of either *Salmonella*, *E. coli* or *L. monocytogenes*. For inactivation studies, single melons were placed in a treatment chamber and exposed to 10mg/L chlorine dioxide gas for 3min. For shelf life trails, melons were exposed to the same conditions but in a pilot scale industrial treatment tunnel and then wrapped and stored at 25˚C for 21 days. As demonstrated by Mahmoud et al. (2008), *Salmonella* was the most sensitive of the pathogens tested to chlorine dioxide gas. The $\log_{10}$CFU reductions were 4.0, 2.9, and 3.3 for *Salmonella*, *E. coli,* and *L. monocytogenes* respectively. Treatment also significantly reduced mesophilic aerobes, yeasts, and moulds compared to untreated melons. The shelf life of rockmelons was also improved by treatment compared to controls. This is one of the few studies identified in the literature review that also reports the use of a pilot scale treatment system. However, the reductions produced over 3min (3 - 4 $\log_{10}$CFU) are unremarkable and would arguably not repay the cost of implementing the infrastructure for such new sanitising systems.

A single study by Hwang et al. (2017) assessed the sequential application of sodium chlorite and hydrochloric acid to generate chlorine dioxide *in situ* for the inactivation of *Salmonella* and *L. monocytogenes* on rockmelon rind sections. Rockmelons were purchased at retail and 4 cm^2 rind sections were removed. The rind sections were spot inoculated with five-strain cocktails of either *Salmonella* or *L. monocytogenes*. The inoculated rind samples were treated by immersion in 35ml of sodium chlorite (1.6 %) for 10min, then dried for 20min. The rind was then subsequently immersed in 35ml of hydrochloric acid (6mM, i.e., 0.05% solution of 12 M HCl) for 10min, and dried for a further 20min. Only the average initial contamination levels for *Salmonella* were reported (5.6 $\log_{10}$ CFU/cm^2). Reductions of > 5 Log_{10}CFU were reported for sequential treatments for both pathogens. We noted the short attachment time for the initial bacterial inoculum of 20min. However, the entire process required an hour to complete. Therefore, this process would need to be extensively optimised and tested to determine if the process could be undertaken in a much shorter time, such as 2min, that would allow Australian melon producers to achieve production targets and the necessary rate of product throughput. Moreover, the quality effects would also need to be assessed and a longer initial attachment time used.

We identified only two papers that studied the efficacy of chlorine dioxide gas to inactivate *L. monocytogenes* on the rind of rockmelon, and a single paper that assessed the efficacy of sequential application of sodium chlorite and hydrochloric acid to generate chlorine dioxide.

A concentration of 10mg/L chlorine dioxide gas for 3min produced a 3.3 log_{10}CFU reduction of *L. monocytogenes* in comparison to a 2.2 log_{10}CFU reduction for exposure to 5mg/L for 2min.

Given the large capital investment that would be required to implement these systems and the inherent dangers of chlorine dioxide gas, it appears that chlorine dioxide in water may be more effective and safer to generate in-house.

Sequential application of sodium chlorite and hydrochloric acid to generate chlorine dioxide *in situ* on melons produced log_{10}CFU reductions of >5, however required 60min for the entire process, which is not feasible for the industry.

Table 11. Summary of studies assessing the efficacy of chlorine dioxide gas or sequential application of sodium chlorite and hydrochloric acid to generate chlorine dioxide to reduce the population of *L. monocytogenes* and other pathogens on rockmelon surfaces.

Reference	Organism	Attachment time	Initial Log_{10}CFU numbers	Sanitiser	Concentration (ppm)	Contact time (min)	Log_{10}CFU reduction
Mahmoud et al. (2008)	*L. monocytogenes*	1h	~7.5	Chlorine dioxide gas	0.5	2	1.2
Mahmoud et al. (2008)	*L. monocytogenes*	1h	~7.5	Chlorine dioxide gas	1.0	2	1.8
Mahmoud et al. (2008)	*L. monocytogenes*	1h	~7.5	Chlorine dioxide gas	1.5	2	2.1
Mahmoud et al. (2008)	*L. monocytogenes*	1h	~7.5	Chlorine dioxide gas	3.0	2	2.1
Mahmoud et al. (2008)	*L. monocytogenes*	1h	~7.5	Chlorine dioxide gas	5.0	2	2.2
Mahmoud et al. (2008)	*E. coli*	1h	~7.5	Chlorine dioxide gas	0.5	2	0.6
Mahmoud et al. (2008)	*E. coli*	1h	~7.5	Chlorine dioxide gas	1.0	2	1.1
Mahmoud et al. (2008)	*E. coli*	1h	~7.5	Chlorine dioxide gas	1.5	2	1.1
Mahmoud et al. (2008)	*E. coli*	1h	~7.5	Chlorine dioxide gas	3.0	2	2.2
Mahmoud et al. (2008)	*E. coli*	1h	~7.5	Chlorine dioxide gas	5.0	2	2.2
Mahmoud et al. (2008)	*S.* Poona	1h	~7.5	Chlorine dioxide gas	0.5	2	0.9
Mahmoud et al. (2008)	*S.* Poona	1h	~7.5	Chlorine dioxide gas	1.0	2	1.2

Mahmoud et al. (2008)	*S.* Poona	1h	~7.5	Chlorine dioxide gas	1.5	2	1.5
Mahmoud et al. (2008)	*S.* Poona	1h	~7.5	Chlorine dioxide gas	3.0	2	3.2
Mahmoud et al. (2008)	*S.* Poona	1h	~7.5	Chlorine dioxide gas	5.0	2	3.2
Trinetta et al. (2013)	*L. monocytogenes*	1h	6.1	Chlorine dioxide gas	10	3	3.3
Trinetta et al. (2013)	*E. coli*	1h	6.0	Chlorine dioxide gas	10	3	2.9
Trinetta et al. (2013)	*Salmonella*	1h	5.9	Chlorine dioxide gas	10	3	4.0
Hwang et al. (2017)	*L. monocytogenes*	20min	NR	Chlorine dioxide [a]	[a]	60.0	5.2
Hwang et al. (2017)	*Salmonella*	20min	5.6	Chlorine dioxide [a]	[a]	60.0	5.1

[a] Generated by sequential application by immersion in 35ml of sodium chlorite (1.6 %) for 10min, then dried for 20min. The rind was then immersed in 35ml of hydrochloric acid (6mM, i.e., 0.05% solution of 12 M HCl) for 10min, and dried for a further 20min.

8.2.2.2 Hydrogen peroxide

Hydrogen peroxide (aqueous)		
What is the status as a fruit/vegetable sanitiser?	Hydrogen peroxide is a commonly used antimicrobial compound and is used for medical, food and industrial applications.	(Ramos et al., 2013; Parish et al., 2003)
What is it?	Hydrogen peroxide (H_2O_2) is an inorganic compound composed of hydrogen and oxygen and is a strong oxidising agent.	(PubChem, 2020b)
How is it applied?	It can be applied as a wash or spray combined with water.	
How does it work?	Hydrogen peroxide produces hydroxyl free radicals that denature proteins and increase the permeability of bacterial cell membranes.	(Linley et al., 2012)
How effective is it?	It is reported to be broadly antimicrobial, acting against both Gram-negative and positive organisms. Low antimicrobial efficacy at permitted levels for vegetables. Less effective against yeasts, fungi, and viruses.	(Ramos et al., 2013; Parish et al., 2003)
What factors influence effectiveness?	Concentration, temperature. High-concentrations are required to be effective and may reduce the quality of produce. Hydrogen peroxide alone is not suited to industrial scale water or produce sanitation due to slow inactivation kinetics and rapid consumption by organic compounds.	(Van Haute et al., 2015)
Is it approved or regulated?	Hydrogen peroxide is currently permitted for use as a processing aid in the *Australia New Zealand Food Standards Code*. It is a permitted bleaching, washing and peeling agents, for all foods, 5mg/kg maximum permitted level.	(FSANZ, 2016a)
Relative cost?	Low relative cost.	
What plant or process changes might be required?	Minimal, able to be incorporated in current processes.	
Are there environmental considerations?	Streams, rivers, and waterways should not be contaminated with these chemicals or used empty containers. Rapidly breaks down to nontoxic products.	
Are there OH&S considerations?	Safe handling and storage of chemicals. Safe Work Australia exposure standards are documented.	
Other Advantages	Sporicidal. Not corrosive at permitted levels. Easy to use.	(Ramos et al., 2013; Parish et al., 2003)
Other Disadvantages	Phytotoxicity against some products like lettuce and berries Negative impact on overall quality May require the removal of residual hydrogen peroxide after processing High concentrations required for efficacy that are not feasible	(Ramos et al., 2013; Parish et al., 2003)

Our review identified four papers that investigated the ability of hydrogen peroxide to reduce the number of *L. monocytogenes* and other pathogens on the surface of melons. The studies are summarised in Table 12.

Ukuku and Fett (2002), introduced in Section 8.2.1.1, assessed chlorine and 5% hydrogen peroxide on whole rockmelons inoculated by immersion in a four-strain cocktail of *L. monocytogenes*. The methods were the same as those reported for chlorine. Inoculated melons were stored at 4˚C for 24h and then treated with 5% hydrogen peroxide for 2min. Under these conditions, the population of *L. monocytogenes* was reduced by 3.2 Log_{10}CFU. This result was the same as that observed for the very high concentration (and not commercially realistic) of 1000ppm chlorine that was also assessed (*see* Section 8.2.1.1).

Ukuku et al. (2005) aimed to determine the efficacy of hydrogen peroxide (2.5%) alone or hydrogen peroxide in combination with nisin, sodium lactate, and citric acid as potential sanitisers for reducing *E. coli* O157:H7 or *L. monocytogenes* populations on whole rockmelon and honeydew melons. Melons were inoculated by immersion in either a two-strain cocktail of *E. coli* or *L. monocytogenes*. Melons were treated on day 0 or stored at 5˚C for 7 days before treatment to assess the potential influence of biofilm formation. On day 0 and 7, melons were sanitised by immersion in 3L of either 2.5% hydrogen peroxide or a combination of 1% hydrogen peroxide, 25µg/ml nisin, 1% sodium lactate, with 0.5% citric acid (HPLNC) for 5min. Mesophilic aerobes, yeasts and moulds were also enumerated on appropriate media. HPLNC was consistently a more effective sanitiser compared to 2.5% hydrogen peroxide for both melon types and pathogens, reducing *L. monocytogenes* to below the experimental LOD (1.3 $log_{10}CFU/cm^2$) on both melon types and *E. coli* on honeydew melons. Populations of *E. coli* slightly declined and *L. monocytogenes* levels did not change during storage at 5˚C for 7 days.

Ukuku et al. (2012) assessed the effect of hydrogen peroxide (2.5%) or tap water treatment of rockmelon rind surfaces on survival of native microflora and *L. monocytogenes*. Melons purchased at wholesale were inoculated by submerging for 10min in a four-strain *L. monocytogenes* cocktail. The melons were dried and then submerged in either 2.5% hydrogen peroxide, or tap water for 5min. The results demonstrated that washing with water did not produce reductions of > 0.3 log_{10}CFU for *L. monocytogenes,* aerobic mesophiles, or yeasts and moulds, but 2.5% hydrogen peroxide produced 2.8, 2.6, and 2.4 log_{10}CFU reductions for *L. monocytogenes*, aerobic

mesophiles, or yeasts and moulds respectively. This result was similar to the 2.3 log_{10}CFU reduction for 2.5% hydrogen peroxide applied for 5min previously reported (Ukuku et al., 2005).

Four published studies provide evaluation of the efficacy of hydrogen peroxide alone and in combination with other antimicrobials to reduce *L. monocytogenes* and other pathogens on the surface of whole melons.

Log_{10}CFU reductions of 1.8 to > 3.2 for *L. monocytogenes* on the surface of rockmelons have been reported for various concentrations applied for 2 – 5min.

The results for inactivation due to hydrogen peroxide are also unremarkable in comparison to those reported for other sanitisers. Hydrogen peroxide alone is not suitable for rockmelon industry use unless it is catalysed with metals such as silver. However, as mentioned in Ukuku et al. (2012) washing in 2.5% hydrogen peroxide for 5min could reduce the risk to susceptible populations if used in the home or at retail level before processing fresh cut rockmelon.

Table 12. Summary of studies assessing hydrogen peroxide efficacy to reduce the population of *L. monocytogenes* and other pathogens on rockmelon surfaces.

Reference	Organism	Attachment time	Initial Log_{10}CFU numbers	Sanitiser	Concentration (%)	Contact time (min)	Log_{10}CFU reduction
Ukuku and Fett (2002)	*L. monocytogenes*	24h	3.5	Hydrogen peroxide	5	2	> 3.2
Ukuku et al. (2005)	*L. monocytogenes*	1h	4.1	Hydrogen peroxide	2.5	5	2.3
Ukuku et al. (2005)	*L. monocytogenes (HD)*	1h	3.1	Hydrogen peroxide	2.5	5	2.6
Ukuku et al. (2005)	*L. monocytogenes*	1h	4.1	HPLNC	a	5	> 2.8
Ukuku et al. (2005)	*L. monocytogenes (HD)*	1h	3.1	HPLNC	a	5	>1.8
Ukuku et al. (2005)	*E. coli*	1h	5.3	Hydrogen peroxide	2.5	5	3.0
Ukuku et al. (2005)	*E. coli (HD)*	1h	3.5	Hydrogen peroxide	2.5	5	3.0
Ukuku et al. (2005)	*E. coli*	1h	5.3	HPLNC	a	5	4.4
Ukuku et al. (2005)	*E. coli (HD)*	1h	3.5	HPLNC	a	5	> 2.2
Ukuku et al. (2012)	*L. monocytogenes*	2h	4.6	Hydrogen peroxide	2.5	5	2.8

HD = honeydew melon
HPLNC = hydrogen peroxide (1%) in combination with nisin (25 ug/ml), sodium lactate (1%), and citric acid (0.5%)

8.2.2.3 Hot water, steam and other heat treatments

Hot water and types of steam.		
What is the status as a fruit/vegetable sanitiser?	Hot water and different types of steam are widely used in the food industry for sanitation. There is commercial technology available for many applications.	(Xiao et al., 2014; Alfy et al., 2016)
What is it?	Water is not homogenous. It consists of free molecules of water (H_2O) as well as water molecules that are joined together in complex assemblies called clathrates. As temperature is increased, i.e., when water is heated, energy is transferred to water molecules and makes them move faster and more energetically, and more water molecules have enough energy to break free from the clathrates and exist as single water molecules. Eventually when enough heat is added all the hydrogen bonding between water molecules is overcome and they can vaporize (i.e., break free of the rest of the liquid water and form steam). But not all steam is the same and varies depending on the temperature and pressure. **Unsaturated steam** (wet steam) is produced by latent heating of water. Unsaturated steam is visible because it includes some (un-vaporized) water droplets that are big enough to be seen by eye but that still have enough energy to leave the main water phase. These droplets give wet steam its characteristic wetness. **Saturated steam** (dry steam) is formed when water is heated to the boiling point (sensible heating) and then vaporized with additional heat (latent heating). It is formed by further heating of unsaturated steam and is considered 'dry' because it contains only vapourised water molecules (and no water droplets). Thus, saturated steam is invisible. **Superheated steam** is formed by further heating wet or dry steam at a higher temperature (and molecular energy) and has a lower density than saturated steam. Another term used for steam is **Vapour Heat:** Involves the forced supply of heated and saturated water vapour to achieve temperature and humidity distribution.	(TVL, 2020)
How is it applied?	**Hot water** can be used as a dip or wash. **Wet steam** has been applied with hand-held steaming units and in commercial continuous feed steam chambers. **Dry steam** continuous feed systems have also been developed. **Superheated steam** requires a special chamber (to contain the steam) and has only been applied to melons in sealed experimental chambers. **Vapour heat:** Can be applied in sealed chambers or continuous feed. Commercial systems exist.	(Xiao et al., 2014; Alfy et al., 2016)
How does it work?	Heat inactivates microorganisms by causing irreversible coagulation and denaturation of enzymes and structural proteins and other macromolecules (e.g., ribosomes, DNA), because as the vapour or wet steam contacts the cooler sample material they transfer their energy to it, and condense back to water.	
How effective is it?	Hot water and steam is effective against a wide range of microorganisms, but particularly against vegetative cells (such as *L. monocytogenes*). Hotter steam is required to inactivate spore-forming bacteria.	

What factors influence effectiveness?	Temperature, contact time, target organism, other processing parameters.	(Xiao et al., 2014; Alfy et al., 2016)
Is it approved or regulated?	Hot water and steam treatment methods are available commercially in Australia. Consultation with relevant state authorities should be undertaken before use to determine if approval is required.	
Relative cost?	Significant initial investment in equipment. Increased electricity costs to generate hot water/steam.	
What plant or process changes might be required?	Most steam or hot water applications could be incorporated into current processing lines, however, additional equipment is required. Superheated steam would require more extensive re-engineering and considerable cost.	
Are there environmental considerations?	High water use and electricity for steam generation.	
Are there OH&S considerations?	Very hot steam is dangerous to workers (burns) but commercial units provide very good containment to protect workers.	
Other Advantages	Chemical free Well perceived by consumers Potentially highly effective against a range of organisms	(Xiao et al., 2014; Alfy et al., 2016)
Other Disadvantages	Potential for damage to quality of fruit by transfer of heat. Efficacy highly dependent on optimised and well controlled processing parameters A cooling step after treatment is critical to maintaining fruit quality and adds further cost.	(Xiao et al., 2014; Alfy et al., 2016)

We found two studies that assessed the effect of hot water on the reduction of *L. monocytogenes* on the surface of rockmelons. The results are presented in Table 13.

Suslow and Callejas (2015), assessed the efficacy of hot water (63-68˚C) applied via a laboratory thermal shower with and without a subsequent pressure spray application of 30ppm PAA to remove *S*. Typhimurium or *L. innocua* from the surface of whole melons. The same inoculation methods as reported in Section 8.2.1.3. were used. Uninoculated and inoculated melons were either; washed for 45 sec with 63-68˚C water; pressure sprayed for 1 sec with 30 ppm peroxyacetic acid; washed for 45 sec with 63-68˚C water followed by 1 sec pressure spray with 30 ppm PA; or not treated to determine initial populations of bacteria. The results demonstrated that the sequential application of a thermal wash followed by PAA did not produce significant increases in log reductions compared to reductions of 3.4 $\log_{10}$CFU for *S*. Typhimurium and 3.3 for *L. innocua* for the thermal wash alone. PAA applied alone was the least effective treatment in the study (*see* Section 8.2.1.3).

Suslow and Callejas (2015) also assessed the use of a commercial facility thermal shower wash using 65 - 70˚C water followed by spray application of 45-50ppm PAA for unreported application times but are assumed to be similar to those reported above. 1.5, 2.1, and 1.9 Log_{10}CFU reductions for total heterotrophic bacteria, total coliforms, and Enterobacteriaceae (used as an indicator organism), respectively were reported. However, the application of the 50 ppm PAA spray following the thermal shower did not produce increased Log_{10}CFU reductions compared to the thermal shower alone. Melon quality, assessed via weight loss, mould growth, and soft spots, was not negatively affected when stored for 14 days at 2.5˚C. However, some cultivar-specific effects were noted and higher wash temperatures (of 95˚C) produced evidence of increased mould growth likely from melon surface damage. The authors also noted that "it was also demonstrated that these systems have inherent complexities and the potential for negative quality impacts, if the process controls are not stringent, that present some real challenges under commercial system management".

Ukuku et al. (2016b) assessed the efficacy of hot water alone and in combination with hydrogen peroxide to reduce numbers of *L. monocytogenes*, *Salmonella* and *E. coli* O157:H7 on the surface of rockmelons. Rockmelons were purchased at retail and inoculated with two-strain cocktails of either *L. monocytogenes*, *Salmonella* or *E. coli* O157:H7 by immersion of whole melons in 3L of inoculum for 10min. To determine the effect of biofilm formation, melons were then stored for up to 7 days before treatment at 5˚C or 20˚C. On days 0, 3, and 7 melons were treated in either water at 20˚C, 3% hydrogen peroxide at 20˚C, water at 80˚C, or 3% hydrogen peroxide at 80˚C for 5min. The results from day 0 indicate that both water at 80˚C and 3% hydrogen peroxide at 80˚C were more effective than hydrogen peroxide at 20˚C, with all pathogens reduced to below the LOD (not reported by the authors but assumed to be 0.3 Log_{10}CFU). While the authors reported stronger attachment of *Salmonella* and *E. coli* O157:H7 on days 3 and 7 for melons stored at 20˚C there was no effect of attachment time evident in the results for sanitiser treatments for any pathogen. This treatment was applied as an intervention immediately before preparing freshly cut fruit, used a high water temperature of 80˚C, and did not report on quality effects on the whole melons. However, the results indicate that hot water treatments may be effective in inactivating *L. monocytogenes* from the surface of rockmelons. It is surprising that hot water treatments alone at temperatures that may not illicit damage to the fruit have not been investigated further and we suggest that they may warrant further investigation.

Two authoritative studies have assessed the effect of hot water on the reduction of *L. monocytogenes* on the surface of rockmelons.

Log_{10}CFU reductions of > 3 for *L. monocytogenes and L. innocua* on the surface of rockmelons have been achieved for hot water at 65 – 80˚C applied for times from 45s to 5min.

There are limited studies investigating the potential of hot water treatments alone as a sanitisation treatment for *L. monocytogenes* on rockmelon. There may be an increased risk of recontamination following treatment that is not well understood. The most effective systems may be costly and require a good understanding of the inherent complexities.

Hot water may be effective at reducing *L. monocytogenes* on the surface of rockmelons and we suggest that they warrant further investigation.

Table 13. Summary of the published study that has assessed the efficacy of hot water to reduce *L. monocytogenes* and other pathogens on the surface of rockmelons.

Reference	Organism	Attachment time	Initial Log_{10}CFU numbers	Sanitiser	Temperature (˚C)	Contact time (min)	Log_{10}CFU reduction
Suslow and Callejas (2015)	*L. innocua*	76h	3.9	Water	65	0.75	3.3
Suslow and Callejas (2015)	*Salmonella*	76h	3.5	Water	65	0.75	3.4
Suslow and Callejas (2015)	*L. innocua*	76h	3.9	Water + 30ppm PAA spray	65	0.75	2.7
Suslow and Callejas (2015)	*Salmonella*	76h	3.5	Water + 30ppm PAA spray	65	0.75	2.8
Ukuku et al. (2016b)	*L. monocytogenes*	2h	3.6	Water	80	5.0	> 3.3
Ukuku et al. (2016b)	*Salmonella*	2h	4.8	Water	80	5.0	> 4.5
Ukuku et al. (2016b)	*E. coli*	2h	5.1	Water	80	5.0	> 4.8
Ukuku et al. (2016b)	*L. monocytogenes*	2h	3.6	3 % Hydrogen peroxide	20	5.0	2.7
Ukuku et al. (2016b)	*Salmonella*	2h	4.8	3 % Hydrogen peroxide	20	5.0	2.9
Ukuku et al. (2016b)	*E. coli*	2h	5.1	3 % Hydrogen peroxide	20	5.0	4.1
Ukuku et al. (2016b)	*L. monocytogenes*	2h	3.6	3 % Hydrogen peroxide	80	5.0	> 3.3
Ukuku et al. (2016b)	*Salmonella*	2h	4.8	3 % Hydrogen peroxide	80	5.0	> 4.5
Ukuku et al. (2016b)	*E. coli*	2h	5.1	3 % Hydrogen peroxide	80	5.0	> 4.8

> indicates results below the limit of detection

Our review identified six papers that investigated the effect of different types of steam on the survival of *L. monocytogenes* and other pathogens on the surface of rockmelons. The results of the studies are summarised in Table 14.

Kozempel et al. (2002) investigated the application of a vacuum/steam/vacuum (VSV) treatment to reduce *L. innocua* inoculated onto whole rockmelons. A prototype VSV processor was designed and fabricated that allowed for the processing of a single melon that was manually added to the machine. Melons were purchased at retail and immersed in *L. innocua* inoculum. The inoculated melons were processed through the VSV prototype under different processing parameters to determine the optimal conditions for treatment. For a single cycle, the only statistically significant variable was steam temperature. When steam was at 143˚C a 3.5 $\log_{10}$CFU reduction was achieved. The optimum process parameters for a single cycle were initial vacuum time 0.1s, steam 143˚C for 0.1s, and final vacuum time of 0.3s. Two cycles were significantly better than one and achieved a 4.1 $\log_{10}$CFU reduction. To assess whether the formation of biofilms potentially played a role in reducing the efficacy of the treatment, an experiment was undertaken where half the melons were stored in a refrigerator (at an unspecified temperature) for two days before processing. There were no significant differences due to storage time before treatment. There were also no visible signs of thermal damage following treatment. The main limitations of this method are the requirement of significant capital investment for scale up, including the requirement of a closed vessel that would slow continuous production.

Forney et al. (2015) determined the effects of aerated steam treatment on the survival of *L. innocua* on rockmelon surfaces and the effects on melon quality. Rockmelons were obtained from a wholesaler and inoculated by immersion in a four-strain cocktail of *L. innocua*. Melons were treated in a Variable Temperature Thermal Treatment (VT3) System (developed by ABCO Industries Ltd. In collaboration with the Atlantic Food and Horticulture Research Centre) that delivers a precisely controlled aerated steam treatment to fresh produce. This involved a single layer of melons subjected to aerated steam heat at 84˚C for 4min to achieve a melon surface temperature of 64˚C to 74˚C for 2.67min, followed by immediate forced air cooling for an unreported period of time. Following treatment, melons were stored at 4, 7 and 10˚C for up to 14 days. The treatment effect on melon quality and physiology was assessed by visual appearance, respiration rate, fruit volatile production, flesh colour, firmness, soluble solids, and titratable acidity. Although the results from the inactivation studies for *L. innocua* were not reported in

detail, the authors reported that aerated steam of 84˚C for 4min reduced *L. innocua* populations by 4 Log_{10}CFU. However, the treatment caused surface damage to the fruit in terms of surface discoloration, reduced respiration, and altered aroma volatile production but, the colour, firmness, soluble solids and acidity of the edible fruit flesh were unaffected by the heat treatment. The authors suggested that due to the damage caused to the melon rind evident in the rind discoloration and reduced respiration rates, but lack of damage to edible flesh, that this method may be promising for rockmelons to be used as cut fruit.

Ukuku et al. (2016a) investigated the efficacy of wet steam treatments of whole rockmelons to reduce pathogen loads and the effects on melon quality. Rockmelons were purchased from a local distributor and inoculated by immersion in either a two-strain *E. coli* O157:H7, three-strain *Salmonella*, or three-strain *L. monocytogenes* cocktail. The steam generator used was a handheld 915 Power Steamer (Wagner SprayTech Corp. MN). The melons were treated with wet steam with the nozzle 7.6cm from the melon surface to achieve a surface temperature of 68˚C either by a fixed method where treatment was at a targeted spot for 3min or by a sweeping method where the treatment was applied in parallel across the entire surface. The quality of melons following treatment was assessed visually and instrumentally for colour and texture. The steady steam treatment resulted in a 3.6 log_{10}CFU reduction of mesophiles, and 3.8 – 4.4 log_{10}CFU reductions for all pathogens. The sweeping treatment resulted in 2.4 log_{10}CFU reduction of mesophiles, and 3.0 – 3.4 log_{10}CFU log pathogen reductions. The authors suggested that "The extra 1 log inactivation of bacteria from fixed (spot) treatments for 3min could be attributed to a combination of factors; direct steady wet steam treatment, constant impact of steam temperature and pressure at a spot than when the nozzle was moved in a sweeping motion." This reinforces the importance of determining the optimal parameters for specific situations and equipment for steam application to rockmelons to ensure optimal efficacy. Treated rockmelons did not show signs of decay or demised quality after treatments and during storage for 29 days at 5 °C.

Bezanson et al. (2018) reported the results of the assessment of an industry-developed, continuous feed, steam treatment system for the inactivation of *Listeria* on whole rockmelons. Rockmelons imported to California from Mexico were inoculated by immersion in a four-strain *L. innocua* cocktail. Melons were steam treated with the Variable Temperature Thermal Treatment (VT3) pilot scale prototype (first mentioned above by Forney et al. (2015)) by exposure to aerated steam heated to 85˚C for 4min, 1.9min cooling on transfer belt, and 2.58min cooling in a - 4˚C

chamber. Temperature sensors were placed 2mm beneath the rind surface in the top, bottom and two sides of whole melons to determine optimal parameters of cabinet heat/cooling levels and belt speeds. These instrument-laden melons were also spread evenly among test melons without such sensors and recorded the temperature every 10s. Surface temperature of melons was monitored with a hand-held infrared thermometer. Steam was also tested for the carriage of *L. innocua*. Melons were assessed for quality parameters of appearance, texture, flesh colour, juice acidity, total soluble solids (TSS) and respiration. Preliminary studies determined that rind temperatures of 64˚C for 2.7min produced 4-5 log_{10}CFU reductions of *L. innocua*, and were achieved at pilot scale by processing a single layer of melons in the cabinet with steam heated to 85˚C - 90˚C for < 5min. Melon rind temperatures rose from 23.9 to 64˚C within 50 - 170s of entry to the steam chamber then consistently increased to 67.9 to 78.3˚C during the remaining 70 to 190s of steam exposure. Cooling through the - 4˚C chiller reduced the surface temp from 73.2 to 19.6˚C in 4.5min. The internal flesh temperature increased from 8 - 10 to 16 - 24˚C during cooling, then equilibrated with the storage temperature of 7˚C in 13.0h and 4˚C in 12.4h.

Notably, there was discoloration of the rind after treatment, however, there were no changes in flesh colour or firmness. TSS and juice acidity were not significantly different. Respiration was reduced on average by 57%, but other research demonstrated that respiration continues during storage. Treatment for 4min at 85˚C reduced yeasts and moulds and coliforms to below the LOD (2.4 $Log_{10}CFU/cm^2$), TVC was reduced by 3 Log_{10}CFU and *L. innocua* by 3.9 Log_{10}CFU (SD 0.9) based on three trials. The escaping steam and condensates tested were negative for *L. innocua*. Surviving *L. innocua* did not increase by more than 0.5 Log_{10}CFU during subsequent storage at 4, 7 or 10˚C for 14 days. However, when *L. innocua* was inoculated onto the surface of heat-treated melons to imitate post-treatment contamination and stored at 7˚C for 10 days, *L. innocua* increased by 3.3 log_{10}CFU. The authors also reinoculated non heat-treated melons with *L. innocua* and did not see the same increases as on inoculated heat-treated melons and suggest that the higher populations of native microflora provide a competitive interaction that results in lower *L. innocua* growth. Therefore, melons treated with this method may be more susceptible to recontamination after processing. While this is one of the few methods that has been upscaled to a pilot level and can be incorporated into post-processing, the pilot system still only processed 330 melons per hour which is well below some industry targets. Moreover, the time required to go through the entire process is 4min in addition to the time on the transfer belt and in the -4˚C cooling cabinet which was a

total of 8.48min. Finally, due to the discoloration caused on the rind, as reported by the authors, this method may only be appropriate for melons destined to become cut fruit.

Kwon et al. (2018) aimed to examine the difference in the ability of saturated steam (SS) and superheated steam (SHS) to reduce *L. monocytogenes* on watermelon and rockmelon surfaces. Melons were purchased from grocery stores and 10cm^2 rind sections were removed and then inoculated with either a three-strain cocktail of *E. coli* O157:H7, *L. monocytogenes* or *S*. Typhimurium. A steam generator apparatus converted water into steam using an electrical resistance heater and required a sealed treatment chamber. Rockmelon rind sections were spread in a single layer in the treatment chamber and exposed to SS or SHS for 5, 10, 15, 20, 25 or 30 s, and watermelon rind for 1, 3, 5, 7 or 10 s. SS treatments were conducted at 100˚C, and SHS treatments were performed at 150 and 200˚C. Surface roughness, colour, and texture were also assessed following treatment. The results demonstrated ~5 $\log_{10}$CFU reductions for *L. monocytogenes*, *E. coli,* and *Salmonella* on both rockmelon and watermelon treated with SHS at 200˚C for 30s and 10s respectively. No negative effects on quality were reported, though colour did vary between samples but not significantly. SHS provided an effective means of reducing populations of pathogens and could produce 5 $\log_{10}$CFU reductions, but rockmelons required 30s to achieve that reduction compared to 10s for watermelon. This could be a promising technology, however, scale up and experiments that assess whole melons are required and a cooling step would also need to be incorporated into the process to prevent prolonged exposure to heat and melon quality degradation.

Kwon et al. (2019) further studied the efficacy of SS and SHS in combination with lactic acid to reduce populations of *L. monocytogenes*, *E. coli* O157:H7 and *S.* Typhimurium on rockmelon surfaces. Melons were purchased from grocery stores and 10cm^2 rind sections were removed and then inoculated with either a three-strain cocktail of *E. coli* O157:H7, *L. monocytogenes* or *S.* Typhimurium. For treatment, rockmelon rind pieces were first immersed or sprayed with distilled water or 2% lactic acid (pH 2.12) for 1min (22˚C). Rind sections were then spread in a single layer in the steam treatment chamber and exposed to SS or SHS on the rind surface for 5, 10, 15, or 20s. SS treatments were conducted at 100˚C, and SHS treatments were performed at 150 and 200˚C. Colour and texture were all assessed after treatment. In the results, all three pathogens were reduced to below the LOD (<1 $\text{Log}_{10}\text{CFU/cm}^2$) following a combination treatment of 2% lactic acid combined with SHS at 200˚C for 20s. No significant differences between immersion or spraying

with lactic acid were found. Following treatment, colour and firmness did not differ between treated samples and controls, however, only rinds and not whole melons were tested. This treatment also seems to have good potential but requires scale up and also needs to incorporate a cooling step and be tested on whole melons. The energy cost and the safety of generating and using superheated steam would also be a consideration.

Six papers were identified that investigated the effect of different types of steam on the survival of *L. monocytogenes* and other pathogens on the surface of rockmelons.

Log_{10}CFU reductions of 3 - 5 for *L. monocytogenes* on the surface of rockmelons have been achieved for various steam applications including pilot scale systems. These represent some of the most effective inactivation methods (e.g., largest log_{10}CFU reductions) identified in our review, and warrant further investigation.

Wet steam, dry steam and vapour heat systems are commercially available, however individual systems may not have been validated for inactivation of *L. monocytogenes*. The application of super-heated steam is still in the R&D stage and has not been assessed on whole melons. There have been reported quality issues from the use of steam including the unacceptable discoloration of the rind.

The results indicate steam and hot water vapour may be effective at reducing *L. monocytogenes* on the surface of rockmelons. However, specifying parameters that do not lead to fruit damage will be essential. There may be an increased risk from recontamination following steam treatment that is not well understood. The systems may be costly and require a good understanding of the inherent complexities before implementation.

Table 14. Summary of published studies that assessed the efficacy of steam to reduce *L. monocytogenes* and other pathogens on the surface of rockmelons.

Reference	Organism	Attachment time	Initial Log_{10}CFU numbers	Sanitiser	Steam temperature (˚C)	Contact time (min)	Log_{10}CFU reduction
Kozempel et al. (2002)	*Listeria innocua*	1h	5.6	VSV	143	0.017	4.1
Kozempel et al. (2002)	*Listeria innocua*	1h	5.4	VSV	143	0.0083	3.5
Forney et al. (2015)	*Listeria innocua*	18 – 20h	5.0	Aerated steam	84	4	4.0
Ukuku et al. (2016a)	*E. coli* O157:H7	24h	4.8	Wet steam #	68	3	4.4
Ukuku et al. (2016a)	*Salmonella*	24h	4.5	Wet steam #	68	3	4.1
Ukuku et al. (2016a)	*L. monocytogenes*	24h	4.1	Wet steam #	68	3	3.8
Ukuku et al. (2016a)	*E. coli* O157:H7	24h	4.8	Wet steam	68	3	3.4
Ukuku et al. (2016a)	*L. monocytogenes*	24h	4.1	Wet steam	68	3	3.3
Ukuku et al. (2016a)	*Salmonella*	24h	4.5	Wet steam	68	3	3.0
Bezanson et al. (2018)	*Listeria innocua*	18 – 20h	5.3	Aerated steam	85	4	3.9
Kwon et al. (2018)	*S.* Typhimurium	1	6.01	SS	100	0.5	3.9
Kwon et al. (2018)	*L. monocytogenes*	1	5.99	SS	100	0.5	3.6
Kwon et al. (2018)	*E. coli* O157:H7	1	6.72	SS	100	0.5	2.9
Kwon et al. (2018)	*E. coli* O157:H7	1	6.72	SHS	200	0.5	5.3
Kwon et al. (2018)	*S.* Typhimurium	1	6.01	SHS	200	0.5	5.0
Kwon et al. (2018)	*L. monocytogenes*	1	5.99	SHS	200	0.5	5.0
Kwon et al. (2018)	*S.* Typhimurium	1	6.01	SHS	150	0.5	4.7
Kwon et al. (2018)	*E. coli* O157:H7	1	6.72	SHS	150	0.5	4.3
Kwon et al. (2018)	*L. monocytogenes*	1	5.99	SHS	150	0.5	4.0
Kwon et al. (2018)	*S.* Typhimurium (WM)	1	5.99	SS	100	0.16	3.5
Kwon et al. (2018)	*E. coli* O157:H7 (WM)	1	6.16	SS	100	0.16	3.5
Kwon et al. (2018)	*L. monocytogenes* (WM)	1	5.89	SS	100	0.16	3.1
Kwon et al. (2018)	*E. coli* O157:H7 (WM)	1	6.16	SHS	200	0.16	5.2
Kwon et al. (2018)	*S.* Typhimurium (WM)	1	5.99	SHS	200	0.16	5.0
Kwon et al. (2018)	*L. monocytogenes* (WM)	1	5.89	SHS	200	0.16	4.9
Kwon et al. (2018)	*S.* Typhimurium (WM)	1	5.99	SHS	150	0.16	4.4
Kwon et al. (2018)	*E. coli* O157:H7 (WM)	1	6.16	SHS	150	0.16	4.1
Kwon et al. (2018)	*L. monocytogenes* (WM)	1	5.89	SHS	150	0.16	3.7

Kwon et al. (2019)	*L. monocytogenes*	1	NR	LA + SHS	150[a]	1.34	4.7
Kwon et al. (2019)	*E. coli* O157:H7	1	6.52	LA + SHS	150[a]	1.34	4.6
Kwon et al. (2019)	*E. coli* O157:H7	1	6.52	LA + SHS	150[a]	1.34	4.3
Kwon et al. (2019)	*L. monocytogenes*	1	NR	LA + SHS	150[a]	1.34	4.1
Kwon et al. (2019)	*S*. Typhimurium	1	NR	LA + SHS	150[a]	1.34	4.1
Kwon et al. (2019)	*S*. Typhimurium	1	NR	LA + SHS	150[a]	1.34	4.0
Kwon et al. (2019)	*E. coli* O157:H7	1	6.52	LA + SHS	200[b]	1.34	5.5
Kwon et al. (2019)	*L. monocytogenes*	1	NR	LA + SHS	200[b]	1.34	5.4
Kwon et al. (2019)	*L. monocytogenes*	1	NR	LA + SHS	200[b]	1.34	5.4
Kwon et al. (2019)	*S*. Typhimurium	1	NR	LA + SHS	200[b]	1.34	5.3
Kwon et al. (2019)	*E. coli* O157:H7	1	6.52	LA + SHS	200[b]	1.34	5.2
Kwon et al. (2019)	*S*. Typhimurium	1	NR	LA + SHS	200[b]	1.34	4.9
Kwon et al. (2019)	*L. monocytogenes*	1	NR	LA + SS	100[c]	1.34	4.1
Kwon et al. (2019)	*E. coli* O157:H7	1	6.52	LA + SS	100[c]	1.34	3.8
Kwon et al. (2019)	*L. monocytogenes*	1	NR	LA + SS	100[c]	1.34	3.8
Kwon et al. (2019)	*E. coli* O157:H7	1	6.52	LA + SS	100[c]	1.34	3.5
Kwon et al. (2019)	*S*. Typhimurium	1	NR	LA + SS	100[c]	1.34	3.5
Kozempel et al. (2002)	*Listeria innocua*	1h	5.6	VSV	143	0.017	4.1
Kwon et al. (2019)	*E. coli* O157:H7	1	6.52	LA + SS	100[c]	1.34	3.8
Kwon et al. (2019)	*L. monocytogenes*	1	NR	LA + SS	100[c]	1.34	3.8
Kwon et al. (2019)	*E. coli* O157:H7	1	6.52	LA + SS	100[c]	1.34	3.5
Kwon et al. (2019)	*S*. Typhimurium	1	NR	LA + SS	100[c]	1.34	3.5

WM = Watermelon
VSV = Vacuum/steam/vacuum
SS = Saturated steam
SHS = Superheated steam
LA = Lactic acid
NR = Not reported
# = steady spray treatment
[a] Lactic acid 2% 2.12 pH for 1min + SHS 150˚C 20s
[b] Lactic acid 2% 2.12 pH for 1min + SHS 200˚C 20s
[c] Lactic acid 2% 2.12 pH 1min + SS 100˚C 20s

8.2.2.4 Levulinic acid and sodium dodecyl sulfate

Levulinic acid and sodium dodecyl sulfate		
What is the status as a fruit/vegetable sanitiser?	Levulinic acid is a food grade preservative and flavour ingredient. Sodium dodecyl sulfate (also known as sodium lauryl sulfate) is used as a multipurpose food additive and detergent. The potential use of these GRAS chemicals in combination as a produce sanitiser has been investigated.	(Zhou et al., 2019)
What is it?	Levulinic acid ($C_5H_8O_3$) is a crystalline keto acid. Sodium dodecyl sulfate ($C_{12}H_{25}NaO_4S$) is an organic sodium salt.	(PubChem, 2020e)
How is it applied?	Research has investigated their application as a combined wash or spray or injection, and with potential pre- and post-harvest applications. Applied as a solution.	
How does it work?	Levulinic acid and sodium dodecyl sulfate are reported to complement each other. Levulinic acid increases cell permeability allowing more sodium dodecyl sulfate to enter and damage the cell. Sodium dodecyl sulfate can denature proteins and antimicrobial effects are increased at pH of 1.5 - 3.0.	(Zhou et al., 2019)
How effective is it?	Reported to have limited efficacy separately but combined are active against a range of bacteria and viruses. Differing reports of efficacy on produce.	(Zhou et al., 2019)
What factors influence effectiveness?	To be determined. Efficacy not limited by organic matter.	(Zhou et al., 2019)
Is it approved or regulated?	Requires consultation with state authorities, APVMA and/or FSANZ before use regarding requirements for approvals. Sodium lauryl sulphate is listed as a generally permitted processing aid by FSANZ.	
Relative cost?	An estimate for 3% levulinic acid preparation is 20 cents per litre. It is produced from cellulose-containing waste material. Sodium dodecyl sulfate is widely produced. Potentially low cost.	(Zhou et al., 2019)
What plant or process changes might be required?	As a wash, it would be able to be easily incorporated into existing sanitising systems.	
Are there environmental considerations?	Streams, rivers, and waterways should not be contaminated with these chemicals nor used empty containers.	
Are there OH&S considerations?	Safe handling and storage of chemicals.	
Other Advantages	Potentially non-corrosive to equipment, but acids used to achieve low pH may be.	(Zhou et al., 2019)
Other Disadvantages	Further R&D still required.	

Webb et al. (2015) aimed to assess the efficacy of levulinic acid (LVA) and sodium dodecyl sulfate (SDS) applied in single or double hurdle applications to reduce *L. monocytogenes* on whole melons. Fresh-harvested rockmelons were obtained from growers prior to washing or packing. For the dump tank simulation, the same inoculation methods as for chlorine treatment were used (*see* Section 8.2.1.1) and inoculated melons were treated by immersion in 10L of 5%LVA + 2.0% SDS for 8min. For the single hurdle dip treatment, inoculated melons were treated by immersion in 4L 5.0%LVA + 2.5%SDS for 1min. For the double hurdle treatment, melons were first dump tanked for 10min in 200ppm chlorine then dipped for 1min in 5.0%LVA + 2.5%SDS. The results (Table 15) showed that the single hurdle treatment and double hurdle treatment were equally effective and reduced *L. monocytogenes* on the rind to below the LOD (which was not reported but is assumed as 1.3 $\log_{10}$CFU). However, the dump tank treatment with 5%LVA + 2.0% SDS for 8min only produced a 2.4 $\log_{10}$CFU reduction. This could potentially be due to the lower concentration of SDS. The authors also reported results from inoculation of the stem scar area (not shown) and found that single and double hurdle treatments were more effective at inactivating *L. monocytogenes* inoculated onto the rind then on the stem scar. A double hurdle sanitising process that takes > 5min is unlikely to be feasible for the industry, particularly when these results indicate that the use of 5.0%LVA + 2.5%SDS and dipping for 1min produced comparable results. However, given that a small reduction in SDS when dump tanked for 8min did not produce similar inactivation levels, further research would be required to determine the reproducibility of that result.

One study assessed the efficacy of levulinic acid (LVA) and sodium dodecyl sulfate (SDS) applied in single or double hurdle applications to reduce *L. monocytogenes* on whole melons

Log_{10}CFU reductions of 2.4 to > 3.1 for *L. monocytogenes* on the surface of rockmelons were reported for different treatments bur further research is required to confirm some of the results.

Table 15. Summary of the single published study that has assessed the efficacy of levulinic acid (LVA) and sodium dodecyl sulfate (SDS) in combination to reduce *L. monocytogenes* on the surface of rockmelons.

Reference	Organism	Attachment time	Initial Log_{10}CFU numbers	Sanitiser	Concentration	Contact time (min)	Log_{10}CFU reduction
Webb et al. (2015)	*L. monocytogenes*	18	4.4	LVA + SDS	a	8	2.4
Webb et al. (2015)	*L. monocytogenes*	18	4.4	LVA + SDS	b	1	> 2.1
Webb et al. (2015)	*L. monocytogenes*	18	4.4	Chlorine then LVA + SDS	c	11	> 2.1

a – single hurdle dump tank simulation 8min 5.0%LVA + 2.0%SDS
b – single hurdle dip treatment 1min 5.0%LVA + 2.5%SDS
c – double hurdle 200ppm chlorine dump tank 10min followed by 1min dip 5.0%LVA + 2.5%SDS
> indicates results below the LOD that was not reported but assumed as 1.3 log_{10}CFU

8.2.2.5 Lactic acid wash

Lactic acid wash		
What is the status as a fruit/vegetable sanitiser?	Lactic acid (and other organic acids)s have been investigated as potential sanitisers for fresh produce. They are often used as bio-preservatives in food. Lactic, acetic and citric acid are all GRAS.	(Ölmez and Kretzschmar, 2009)
What is it?	Lactic acid ($C_3H_6O_3$) is a weak organic acid.	(PubChem, 2020d)
How is it applied?	Wash, or in combination with other sanitisers.	
How does it work?	Lactic acid works by reducing pH and damaging the cell membrane of microorganisms and can cause cytoplasmic leakage. At low pH it penetrates the cells and acidifies the cytoplasm causing inactivation.	(Wang et al., 2015)
How effective is it?	Generally long exposure times are required for large reductions on produce. Relatively lower antimicrobial efficacy.	(Ramos et al., 2013; Parish et al., 2003)
What factors influence effectiveness?	Contact time, temperature, the organism itself. Only effective at low pH.	(Ölmez and Kretzschmar, 2009)
Is it approved or regulated?	Lactic acid is an approved food additive. It's use alone and in combinations with other chemicals should be discussed with state authorities before use regarding requirements for approvals.	
Relative cost?	Low relative cost.	
What plant or process changes might be required?	Able to be incorporated into current systems.	
Are there environmental considerations?	Lactic acid use will produce higher COD and BOD in wastewater. Streams, rivers, and waterways should not be contaminated with these chemicals or with used empty containers.	(Ölmez and Kretzschmar, 2009)
Are there OH&S considerations?	Generic safe handling and storage of chemicals. Skin and eye irritants; low pH may be corrosive.	
Other Advantages	Easy to use and apply.	(Ramos et al., 2013; Parish et al., 2003)
Other Disadvantages	Limited reported efficacy alone. Potential for acid resistance in *L. monocytogenes*	

We identified one published study that has evaluated the use of lactic acid alone as a sanitiser for melon surface decontamination for *L. monocytogenes*. Singh et al. (2018), introduced in Section 8.2.1.1, reported on the effectiveness of a number of sanitisers, including a 2% lactic acid wash (initial concentration diluted and subsequent corresponding pH not reported), against *L. monocytogenes* and *Salmonella* on the surface of whole rockmelons. The results are presented in Table 16. While the lactic acid wash demonstrated a > 3 log_{10}CFU reduction of *S.* Typhimurium with a contact time of 5min, only a 2.5 log_{10}CFU reduction was demonstrated for *L. monocytogenes* for the same contact time. Lactic acid was not the most effective sanitiser assessed for either pathogen in the study. As demonstrated in the above sections, acid washes may have an application as part of hurdle technology, however, this area is understudied and not well-documented in the literature.

One study assessed the efficacy of lactic acid as a wash to reduce *L. monocytogenes* on whole melons.

Log_{10}CFU reductions of 2.5 for *L. monocytogenes* on the surface of rockmelons were reported for a 2% concentration wash applied for 5min. There are limited studies at industry relevant contact times and concentrations.

Table 16. Summary of the single published study identified that assessed the efficacy of lactic acid to reduce *L. monocytogenes* and other pathogens on the surface of rockmelons.

Reference	Organism	Attachment time	Initial Log_{10}CFU numbers	Sanitiser	Concentration (%)	Contact time (min)	Log_{10}CFU reduction
Singh et al. (2018)	*S.* Typhimurium	Overnight	7.9	Lactic acid	2	5	3.7
Singh et al. (2018)	*L. monocytogenes*	Overnight	8.8	Lactic acid	2	5	2.5

8.2.2.6 Octenidine dihydrochloride wash

Octenidine dihydrochloride		
What is the status as a fruit/vegetable sanitiser?	Octenidine dihydrochloride has received very limited research into it's potential as a fresh produce sanitiser. It. is currently used in commercial mouth washes/rinses in Europe, and as a skin antiseptic.	(Szostak et al., 2018)
What is it?	Octenidine dihydrochloride ($C_{36}H_{64}C_{l2}N_4$) is a cationic bipyridine.	(Hubner et al., 2010)
How is it applied?	Research to date has investigated wash and coating applications.	
How does it work?	Octenidine dihydrochloride destabilises the membrane of microorganisms.	(Szostak et al., 2018)
How effective is it?	Broad spectrum activity against microorganisms.	(Szostak et al., 2018)
What factors influence effectiveness?	Chemically stable across temperatures, pH 1.6 – 12.2 Not sensitive to light Limiting factors are yet to be determined	(Szostak et al., 2018)
Is it approved or regulated?	Will require consultation with APVMA and/or FSANZ before use regarding requirements for approvals.	
Relative cost?	Very expensive compared to chlorine and other routinely used chemicals.	
What plant or process changes might be required?	If used as a wash would be easily incorporated into existing systems. If used as a coating, significant changes of process would be required.	
Are there environmental considerations?	No information is available regarding the ecotoxicity.	
Are there OH&S considerations?	Does not require special handling or storage measures.	
Other Advantages	Ease of use. Stable and non-reactive.	
Other Disadvantages	Cost. Octenidine dihydrochloride is reported to have an unpleasant taste, however, new formulations may be of interest. Organoleptic effects and quality effects have not been determined. Further R&D is required.	(Szostak et al., 2018)

Upadhyay et al. (2016) assessed the efficacy of an octenidine dihydrochloride wash to reduce *L. monocytogenes*, *E. coli* and *Salmonella* on rockmelon surfaces. Whole melons purchased at retail were inoculated by immersion in 3L of a four-strain cocktail or either *L. monocytogenes*, *E. coli* and *Salmonella*. Whole melons were then immersed in 3L of water or octenidine dihydrochloride (0.1 and 0.2%) for 5min. Rind plugs were removed and enumerated. The results (Table 17) showed that both concentrations reduced the populations of pathogens by > 4.5 log_{10}CFU (LOD of 2 log_{10}CFU). However, the 5min contact time may still be too long for practical application by the industry. Although the authors did not test whole melon sanitisation at contact times less than 5min, rind plug experiments were undertaken at 1, 3, and 5min. In these experiments, rind plugs were removed (2.5cm diameter) from rockmelons and flesh removed from the rind. Each rind plug was spot inoculated with four-strain cocktails of either *L. monocytogenes*, *E. coli*, or *Salmonella* then dried for 2h (25˚C). Individual ring plugs were put in stomacher bags with either sterile deionised water or octenidine dihydrochloride (0.01, 0.05, and 0.10%) and put in a shaking water-bath at 25˚C for 1, 3, 5min. The results showed that for washes with 0.05% and 0.1% octenidine dihydrochloride all pathogens at all time points were reduced to below the LOD with reductions of > 5.2 log_{10}CFU (except *E. coli* with 0.05% octenidine dihydrochloride at 1min with a reduction of 4.2 log_{10}CFU). Therefore, further research may be warranted to assess the efficacy of this sanitiser at contact times more relevant to the industry. Moreover, analysis of the effect of pH, organic matter, and temperature on the effectiveness of this compound would be required to ensure that all optimal conditions for use are also identified.

One study assessed the efficacy of an octenidine dihydrochloride wash to reduce *L. monocytogenes*, *E. coli* and *Salmonella* on rockmelon surfaces.

Log_{10}CFU reductions of >3.6 for *L. monocytogenes* on the surface of rockmelons were reported for 0.1 – 0.2% octenidine dihydrochloride applied for 5min.

Evidence suggests shorter contact times may also achieve high levels of inactivation. Octenidine dihydrochloride is potentially an effective means to reduce *L. monocytogenes*, *E. coli* and *Salmonella* on rockmelon surfaces.

Octenidine dihydrochloride is currently an expensive chemical which may preclude its practical use for rockmelons. Furthermore, there are no data for organoleptic consequences nor effects on shelf life. Further research of the effect of pH, organic matter, and temperature on the effectiveness of this compound would be required.

Table 17. The single study that assessed the efficacy of octenidine dihydrochloride wash to reduce *L. monocytogenes* and other pathogens on the surface of rockmelons.

Reference	Organism	Attachment time	Initial Log_{10}CFU numbers	Sanitiser	Concentration (%)	Contact time (min)	Log_{10}CFU reduction
Upadhyay et al. (2016)	*E. coli*	2h	5.6	Octenidine dihydrochloride	0.1	5.0	4.5
Upadhyay et al. (2016)	*Salmonella*	2h	5.6	Octenidine dihydrochloride	0.1	5.0	4.5
Upadhyay et al. (2016)	*L. monocytogenes*	2h	5.6	Octenidine dihydrochloride	0.1	5.0	> 3.6
Upadhyay et al. (2016)	*E. coli*	2h	5.6	Octenidine dihydrochloride	0.2	5.0	> 3.6
Upadhyay et al. (2016)	*L. monocytogenes*	2h	5.6	Octenidine dihydrochloride	0.2	5.0	> 3.6
Upadhyay et al. (2016)	*Salmonella*	2h	5.6	Octenidine dihydrochloride	0.2	5.0	> 3.5

8.2.2.7 Antimicrobial Coatings

Antimicrobial coatings		
What is the status as a fruit/vegetable sanitiser?	Edible antimicrobial coatings or films are being developed and investigated to extend the shelf life and deliver antimicrobials to fresh produce. There are commercial edible coatings used already in the fresh produce industry to increase shelf life of products, however, their use specifically as a sanitising agent to reduce microbial loads is being developed.	(Dhall, 2013)
What is it?	Edible antimicrobial coatings or films generally consist of a polysaccharide, protein, lipid, or composite matrix, such as chitosan (derived from chitin - a naturally abundant biopolymer) or alginate (cell wall components of brown micro-algae), that have antimicrobials such as essential oils, organic acids, organic acids, fatty acid esters, polypeptides, plant essential oils, nitrites and sulphites incorporated into them	(Dhall, 2013)
How is it applied?	Coatings are required to be heated to make them liquid to allow for dipping of produce and then drying is required.	(Dhall, 2013)
How does it work?	The mode of action against microorganisms will be dependent on the antimicrobial used.	
How effective is it?	This is dependent on the antimicrobial used.	
What factors influence effectiveness?	The antimicrobial used, the polymer matrix used, evenness of coating application and effectiveness of the drying process, and the produce to be coated.	
Is it approved or regulated?	Requires consultation with state authorities, APVMA and/or FSANZ before use regarding requirements for approvals.	
Relative cost?	This will depend on the coating ingredients but some estimates of 1 cent per melon.	(Zhang et al., 2015)
What plant or process changes might be required?	The application of antimicrobial coatings would require significant process changes including the addition of a coating stage and would also require a drying stage before packing.	
Are there environmental considerations?	Dependent on coating used, but could increase BOD and COD in effluents.	
Are there OH&S considerations?	Dependent on coating used.	
Other Advantages	Coatings limit the diffusion of normally volatile antimicrobial compounds compared to washes. May provide protection against recontamination by pathogens. Reduces rate of respiration and delays senescence maintaining quality.	(Dhall, 2013) (Ramos et al., 2013; Parish et al., 2003)
Other Disadvantages	Drying of coating required before packing, hence increasing processing time. Typically composed of products that can elicit allergic reactions. Potential sensory issues with addition of aromatic compounds. Still in R&D phase.	(Dhall, 2013) (Ramos et al., 2013; Parish et al., 2003)

We identified four published studies that have investigated the effect of antimicrobial coatings on reducing *L. monocytogenes* from the surface of rockmelons. The studies that investigated antimicrobial coatings are summarised in Table 18. Because antimicrobial coatings remain on the product an additional column in the summary table (Table 18) describes the growth or suppression of pathogens through storage.

Zhang et al. (2015) assessed the effects of sodium alginate coatings with cinnamon oil (CO) and soybean oil (SO) on the survival of pathogens on rockmelon rinds, and also assessed the effect on quality. Rockmelons were purchased at retail and 6.25cm^2 areas on whole rockmelons were spot inoculated with a five-strain cocktail of either *E. coli*, *L. monocytogenes* or *S. enterica*. Following inoculation, 0.8mL of coating (either 1% w/v alginate, 0.5% w/v Tween® 80, 0 or 2% w/v CO, and 0 or 0.5% w/v SO) was applied with a brush. Melons were then incubated at ambient temperature for up to 15 days. For quality assessment, uninoculated whole melons were dipped in 2L of coating for 10s then dried at 21˚C for 24h and stored at ambient temperature. Weight loss, rind colour, firmness, total soluble solids and yeast and moulds were assessed. The results demonstrated that 24h after coating, the coatings with CO or CO+SO had reduced the levels of all pathogens to below the LOD (1.3 Log_{10} CFU/cm^2). Only melons coated with CO+SO suppressed levels of pathogens to below the LOD for the entire 15 days of storage for all pathogens, and melons coated with only CO had detectable *E. coli* and *L. monocytogenes* after day 7: this was attributed to declining levels of the volatile compounds in the coatings during storage. Alginate only coatings facilitated increased survival compared to controls in some cases for all pathogens and the authors attributed this to the ability of the coating to bind water. Weight loss and total soluble solids content of the flesh were not significantly different between untreated and treated melons, and colour and firmness were retained for longer by the CO+SO treated melons. The authors estimated the cost of coating each rockmelon, including ingredient and operational costs, with alginate and CO+SO coatings to be ~1 (US) cent. A limitation of this study is that it is questionable whether the method of enumeration (rind sections were removed and placed in bags with 25ml diluent and hand rubbed for 1min) may have exposed the bacteria to residual levels of CO/SO that could suppress growth of bacteria on agar plates even if they were, in fact, present and not inactivated.

Upadhyay et al. (2016), introduced in Section 8.2.1.1, also investigated the use of octenidine dihydrochloride in chitosan coatings to reduce populations of *L. monocytogenes, Salmonella,* and *E. coli* on rockmelons. The same inoculation methods as in Section 8.2.1.1 were used. Whole

inoculated rockmelons were coated with only chitosan (2%) or chitosan and octenidine dihydrochloride (0.1 or 0.2%) by dipping them in 3L of the coating treatment (at 25˚C), and dried for 2h. Rind plugs were then removed and stored for up to 3 days at 4˚C. Octenidine dihydrochloride coatings on rind plugs reduced *Salmonella* and *L. monocytogenes* to below the LOD (1.3 $\log_{10}$CFU/cm^2) and these levels were maintained throughout storage. However, reductions of only ~2 $\log_{10}$CFU were recorded for *E. coli* for both octenidine dihydrochloride coatings, which was not as effective as the reductions those authors observed using an octenidine dihydrochloride as a wash in Section 8.2.2.6.

Ma et al. (2016) assessed the effect of chitosan coatings with the addition of lauric arginate (LAE), cinnamon oil (CO), and ethylenediaminetetraacetic acid (EDTA) on the inactivation of *L. monocytogenes*, *Salmonella* and *E. coli* O157:H7 on whole rockmelons. The effect on quality parameters of colour, weight loss, firmness, and total soluble solids was also assessed. The rockmelons were purchased at retail and 6cm^2 areas on rockmelons were inoculated with a five-strain cocktail of either *E. coli, Salmonella* or *L. monocytogenes*. 400ml of coatings of either A: 1% chitosan + 0.1% LAE + 0.1% EDTA; B: coating "A" + 0.5% CO, C: coating "A" + 1% CO; and D: 1% chitosan only, were applied with a brush, and melons stored at room temperature for up to 14 days. The melons for quality tests were dipped in 2L of the same coatings for 30s and then incubated at 21˚C for up to 14 days. All pathogens were significantly reduced by all coatings after 24h compared to uncoated controls, however, only chitosan coatings of 0.1%LAE + 0.1%EDTA + 1%CO kept all pathogens < 3 $\log_{10}$CFU for 14 days. Coating "C" (0.1%LAE + 0.1%EDTA + 1%CO) also delayed growth of mould and yeasts compared to other coatings. Coatings also delayed colour and firmness degradation compared to controls and did not negatively affect weight loss or total soluble solids. Coating "C" was also the most effective at inactivating pathogens throughout 14 days of storage and reduced all pathogens by ~3 $\log_{10}$CFU or more and kept populations at < 3 Log_{10}CFU throughout storage. One question about this research is whether there would be a difference between painting or dipping melons in coatings and how well the coatings would dry on the surface. Moreover, as discussed earlier, the method of enumeration is questionable, i.e., whether the inhibitory effect of the coating is removed into the rinsate and is still active on the plates used to enumerate the surviving bacteria. Aside from these considerations, coatings containing antimicrobials may be a promising technology as they are able to act on and control pathogens throughout storage.

Boyacı et al. (2019) assessed the ability of eugenol incorporated into zein[13] coatings to reduce populations of *L. innocua* and *E. coli* on rockmelon surfaces. Zein coatings were prepared by dissolving 1.4g in 8.2ml of ethanol and adding 0.4ml glycerol. 2% eugenol was assessed in the melon coatings. Two rockmelon cultivars were purchased at retail and 16cm^2 rind sections were inoculated with either *L. innocua* or *E. coli*. 500µl of coating was then applied to the rind section with a pipette and spread with a rod. Melons were then stored at 10˚C for up to 7 days. For sampling, films were peeled off and the rind sections removed from the melon and the flesh separated. Only the rind was added to the diluent for sampling and not the film. The results were similar for the two different cultivars for both pathogens, thus the results for each cultivar were combined for each pathogen for analysis of the results. Zein-2% eugenol coatings produced log_{10}CFU reductions of 2.39 and 2.11 at day 0, and 3.28 and 2.94 at day 7 for *L. innocua* and *E. coli* respectively during storage at 10˚C.

Four studies have assessed the effect of different antimicrobial coatings on the reduction of *L. monocytogenes* on the surface of rockmelons.

Log_{10}CFU reductions of 0.6 to > 5 for *L. monocytogenes* on the surface of rockmelons have been reported for a variety of coatings containing different active ingredients.

As antimicrobial coatings have the advantage of having a prolonged effect on pathogens, the important question to answer is whether they do actually inactivate pathogens and do not simply supress growth. If *L. monocytogenes* were present on the surface of a rockmelon an antimicrobial coating that only inhibits growth, but that does not inactivate pathogens, will be ineffective as it only requires the transfer of the pathogen from the surface to the fruit pulp to then potentially grow and cause illness. Although large reductions of pathogens were reported for specific coatings, the methods of enumeration in the studies may not be sufficiently rigorous to rule out that pathogens were, in fact, not inactivated on the surface of melons rather than being removed with the film, trapped under the film, or supressed on the growth media by active ingredients that were transferred from the coating during the sampling and enumeration procedure. As such, more research is required to confirm the effectiveness of antimicrobial coatings. Moreover, many of the active ingredients used in the coatings (e.g., essential oils) have some aroma associated with them and may not be acceptable to consumers, given that the aroma of a fresh melon can be a contributing factor to purchase choice. While a promising option that also may reduce water usage, these methods still require significant investment and research to prove their efficacy and feasibility at commercial scale levels.

13 Zein is the principle protein component of corn

Table 18. Studies that assessed the efficacy of antimicrobial coatings to reduce *L. monocytogenes* and other pathogens on the surface of rockmelons.

Reference	Organism	Attachment time (h)	Initial numbers $Log_{10}CFU$	Coating and active ingredients	Initial $Log_{10}CFU$ reduction (24h)	Trend through storage
Zhang et al. (2015)	*L. monocytogenes*	NR*	5.9	Alginate 1.0%	0.6	Slow decline and increase
Zhang et al. (2015)	*E. coli*	NR*	4.2	Alginate 1.0%	0.1	Slow decline
Zhang et al. (2015)	*Salmonella enterica*	NR*	4.6	Alginate 1.0%	0.0	Slow decline
Zhang et al. (2015)	*L. monocytogenes*	NR*	5.9	Cinnamon bark oil 2% + Alginate 1.0%	> 4.6	Growth at day 7
Zhang et al. (2015)	*Salmonella enterica*	NR*	4.6	Cinnamon bark oil 2% + Alginate 1.0%	> 3.3	Maintained (15 days)
Zhang et al. (2015)	*E. coli*	NR*	4.2	Cinnamon bark oil 2% + Alginate 1.0%	>2.9	Growth at day 7
Zhang et al. (2015)	*L. monocytogenes*	NR*	5.9	Cinnamon bark oil 2 % + Soybean oil 0.5% + Alginate 1.0%	> 4.6	Maintained (15 days)
Zhang et al. (2015)	*Salmonella enterica*	NR*	4.6	Cinnamon bark oil 2 % Soybean oil 0.5% + Alginate 1.0%	> 3.3	Maintained (15 days)
Zhang et al. (2015)	*E. coli*	NR*	4.2	Cinnamon bark oil 2 % + Soybean oil 0.5% + Alginate 1.0%	> 2.9	Maintained (15 days)
Upadhyay et al. (2016)	*L. monocytogenes*	2h	5.6	Chitosan 2%	2.2	Slight increase
Upadhyay et al. (2016)	*E. coli*	2h	5.6	Chitosan 2%	0.5	Maintained (3 days)
Upadhyay et al. (2016)	*Salmonella*	2h	5.6	Chitosan 2%	1.0	Increase
Upadhyay et al. (2016)	*L. monocytogenes*	2h	5.6	Octenidine dihydrochloride 0.1% + Chitosan 2%	> 5.0	Maintained (3 days)
Upadhyay et al. (2016)	*Salmonella*	2h	5.6	Octenidine dihydrochloride 0.1% + Chitosan 2%	> 3.9	Maintained (3 days)
Upadhyay et al. (2016)	*E. coli*	2h	5.6	Octenidine dihydrochloride 0.1% + Chitosan 2%	1.7	Slight increases
Upadhyay et al. (2016)	*L. monocytogenes*	2h	5.6	Octenidine dihydrochloride 0.2% + Chitosan 2%	> 4.1	Maintained (3 days)
Upadhyay et al. (2016)	*Salmonella*	2h	5.6	Octenidine dihydrochloride 0.2% + Chitosan 2%	> 3.9	Maintained (3 days)

Upadhyay et al. (2016)	*E. coli*	2h	5.6	Octenidine dihydrochloride 0.2% + Chitosan 2%	2.2	Slight increase
Ma et al. (2016)	*L. monocytogenes*	6h	5.7	LAE 0.1% + EDTA 0.1% + Chitosan 1.0%	3.4	Slight increases
Ma et al. (2016)	*E. coli O157:H7*	6h	5.0	LAE 0.1% + EDTA 0.1% + Chitosan 1.0%	3.2	Increases
Ma et al. (2016)	*Salmonella enterica*	6h	4.2	LAE 0.1% + EDTA 0.1% + Chitosan 1.0%	> 2.6	Maintained (14 days)
Ma et al. (2016)	*L. monocytogenes*	6h	5.7	LAE 0.1% + EDTA 0.1% + CO 0.5% + Chitosan 1.0%	3.1	Increases
Ma et al. (2016)	*E. coli O157:H7*	6h	5.0	LAE 0.1% + EDTA 0.1% + CO 0.5% + Chitosan 1.0%	3.0	Increases
Ma et al. (2016)	*Salmonella enterica*	6h	4.2	LAE 0.1% + EDTA 0.1% + CO 0.5% + Chitosan 1.0%	> 2.6	Maintained (14 days)
Ma et al. (2016)	*L. monocytogenes*	6h	5.7	LAE 0.1% + EDTA 0.1% + CO 1.0% + Chitosan 1.0%	> 4.1	Slight increases
Ma et al. (2016)	*Salmonella enterica*	6h	4.2	LAE 0.1% + EDTA 0.1% + CO 1.0% + Chitosan 1.0%	> 2.6	Maintained (14 days)
Ma et al. (2016)	*E. coli O157:H7*	6h	5.0	LAE 0.1% + EDTA 0.1% + CO 1.0% + Chitosan 1.0%	2.5	Slight increases
Boyacı et al. (2019)	*E. coli*	0.5	4.3	Zein	2.1	Slight increase (7 days)
Boyacı et al. (2019)	*Listeria innocua*	0.5	4.6	Zein	1.8	Slight increase
Boyacı et al. (2019)	*Listeria innocua*	0.5	4.6	Zein + eugenol 2%	2.4	Slight decrease
Boyacı et al. (2019)	*E. coli*	0.5	4.3	Zein + eugenol 2%	2.1	Slight decrease

NR* = not reported
chitosan, lauric arginate (LAE), cinnamon oil (CO), and ethylenediaminetetraacetic acid (EDTA)
> indicates results below the limit of detection

8.2.2.8 Essential oil emulsions

Intervention: Essential oil emulsions		
What is the status as a fruit/vegetable sanitiser?	Extensive research has been undertaken to determine the antimicrobial properties of essential oils as potential replacements for other chemicals. More recent research has focused on the development of essential oil nano-emulsions.	(Calo et al., 2015)
What is it?	Essential oils are plant-derived concentrated hydrophobic mixtures of volatile and aromatic compounds. These compounds consist of major constituents of antimicrobials and trace amounts of other compounds. For example, the major antimicrobial compound in cinnamon oil is cinnamaldehyde that is classified as an aldehyde. Essential oils are volatile and hydrophobic so their stability is limited if just mixed with water, as they will separate and disperse. However, nano-emulsions can be created by adding a soap-like surfactant that creates tiny droplets of essential oil dispersed in water that remain stable.	(Prakash et al., 2018)
How is it applied?	Essential oil nano-emulsions have been applied in research as produce washes or as additions to antimicrobial coatings.	
How does it work?	Essential oil constituents can pass through the cell membrane and inhibit cell functions.	(Calo et al., 2015)
How effective is it?	Different essential oils have been documented to be effective for inactivating a range of microorganisms, but the efficacy is dependent on the type of essential oil.	(Calo et al., 2015)
What factors influence effectiveness?	Type of essential oil, size of the nano droplets, storage time, target organism, concentration, contact time, temperature, pH, organic matter, and produce type.	(Prakash et al., 2018)
Is it approved or regulated?	Requires consultation with state authorities and/or FSANZ before use regarding requirements for approvals.	
Relative cost?	Dependent on essential oil type and method of emulsification.	
What plant or process changes might be required?	To be determined.	
Are there environmental considerations?	Dependent on oil type and active ingredients. Minimise contamination of waterways.	
Are there OH&S considerations?	Safe handling and storage of oils. Also, dependent on oil type and method of emulsification.	
Other Advantages	Customer perception as natural.	(Calo et al., 2015)
Other Disadvantages	Break-down over time Potential organoleptic issues due to aroma or flavour.	(Calo et al., 2015)

Our systematic review process identified two published studies that investigated the use of essential oil emulsions to reduce *L. monocytogenes* and other pathogens on the surface of rockmelons. Table 19 summarises the data from the two studies.

Zhang et al. (2016) sought to characterize the antimicrobial properties of emulsified thyme oil and determine the efficacy of those emulsions to reduce *L. monocytogenes*, *Salmonella* and *E. coli* on rockmelons. Rockmelons were purchased at retail. For rind only experiments, 6.25cm^2 sections of rind were removed and the flesh separated, then washed in sterile water, and dried for 1h (21˚C). Rind sections were then spot inoculated with five-strain cocktails of *E. coli*, *Salmonella* or *L. monocytogenes*. Rind sections were immersed in 20ml solutions containing either 0, 0.1, 0.2 or 0.5% (w/v) non-emulsified, or gum arabic (GA)-emulsified thyme oil, in sterile water for 2min. Rind sections were removed and placed in stomacher bags with 20ml sterile phosphate-buffered saline + 0.2% Tween 80 and hand-rubbed for 1min. For whole melon experiments, melons were spot inoculated with 100µl of five-strain cocktails on four 6.25cm^2 sections and dried overnight. Each melon was immersed in 3L of sterile water, or 0.2% free or GA-emulsified thyme oil for 2min. Melons were stored for up to 10 days at 21˚C. The emulsion prepared with GA was experimentally pre-determined to have small stable droplets and antimicrobial activity. For melon rind, washing with water did not produce significant reductions of pathogens. The thyme oil emulsion was generally more effective than the non-emulsified thyme oil washes. The lowest concentration of emulsified thyme oil (0.1%) reduced all pathogens by approximately 1 $\log_{10}$CFU on rind sections, while the highest level (0.5%) reduced *E. coli*, *L. monocytogenes* and *Salmonella* by 2.3, 1.7 and 3 Log_{10}CFU respectively. There was no significant effect of organic load on the reduction of pathogens. Treatment of whole rockmelons with 0.2% emulsified thyme oil demonstrated a 4.70 and 2.19 Log_{10}CFU reduction for *Salmonella* and *L. monocytogenes* respectively but was not consistent for *L. monocytogenes* across 10 days of storage with higher counts after 2 days of storage which was attributed to the volatisation of the essential oil leading to a lower concentration with reduced antimicrobial efficacy.

Paudel et al. (2019) aimed to determine the efficacy of a cinnamon oil (CO) nano-emulsion against *L. monocytogenes* and *Salmonella* on rockmelon and honeydew melons. CO nano-emulsions were prepared in sterile distilled water and Tween 80 by ultra-sonication. The particle size was measured and the smallest particle size emulsion was then diluted to create three different CO concentrations of 0.1%, 0.25% and 0.5%. Melons were purchased at retail and 2cm^2 sections were

removed and then spot inoculated with either a three-strain cocktail of *L. monocytogenes* or *Salmonella*. The rind sections were then immersed in nano-emulsion treatments for 1min. Treated rind sections were then incubated for 24h, 48h or 72h but at an unspecified temperature. The results indicated that 9.63nm was the average particle size of the emulsion. The initial reductions 24h after treatment (Table 19) were unremarkable except for *L. monocytogenes* on rockmelons for which a 2.9 Log_{10}CFU reduction for 0.5% CO was demonstrated. However, this observation had a large standard deviation and was, unexpectedly, much greater than the reduction for honeydew melons. Following storage for 72h, 0.5% CO compared to the water control, induced a > 7 log_{10}CFU reduction of *L. monocytogenes* and *Salmonella* on rockmelon rind, however this was not significantly different to the water only treatment at 72h suggesting that in this case the CO alone was not responsible for the pathogen reductions observed. The reductions on honeydew melons were much smaller at 72h, i.e., almost no reduction. Limitations in this study were a very short attachment time for the inoculated bacteria (only 15min), large variation in some results, no limit of detection was reported, and no information about the temperature of incubation was provided. Therefore, this study would need to be redesigned and repeated to determine if CO nano-emulsions are reliably effective at reducing *L. monocytogenes* on the surface of rockmelons.

Two published studies have assessed the effect of essential oil emulsions on the reduction of *L. monocytogenes* on the surface of rockmelons.

Log_{10}CFU reductions of 0.2 – 2.9 for *L. monocytogenes* on the surface of rockmelons have been achieved for a variety of essential oil emulsions applied for 1 – 2min.

The anti-listerial efficacy of the reported essential oil emulsions alone appears limited.

Essential oils may change the aroma of the fruit unless specific non-aromatic compounds can be identified: therefore it is unlikely the industry would prefer these given that the smell of a rockmelon is something customers associate with quality.

Table 19. Studies that assessed the efficacy of essential oil emulsions to reduce *L. monocytogenes* and other pathogens on the surface of rockmelons.

Reference	Organism	Attachment time	Initial Log_{10}CFU numbers	Essential oil	Concentration (%)	Contact time (min)	Log_{10}CFU reduction
Zhang et al. (2016)	*E. coli* O157:H7	2.5h	6.6	Thyme oil	0.10	2	1.0
Zhang et al. (2016)	*L. monocytogenes*	2.5h	5.9	Thyme oil	0.10	2	1.0
Zhang et al. (2016)	*Salmonella*	2.5h	6.9	Thyme oil	0.10	2	1.0
Zhang et al. (2016)	*Salmonella*	2.5h	6.9	Thyme oil	0.20	2	4.7
Zhang et al. (2016)	*L. monocytogenes*	2.5h	5.9	Thyme oil	0.20	2	2.2
Zhang et al. (2016)	*Salmonella*	2.5h	6.9	Thyme oil	0.50	2	3.0
Zhang et al. (2016)	*E. coli* O157:H7	2.5h	6.6	Thyme oil	0.50	2	2.3
Zhang et al. (2016)	*L. monocytogenes*	2.5h	5.9	Thyme oil	0.50	2	1.7
Zhang et al. (2016)	*E. coli* O157:H7	2.5h	6.6	Thyme oil	0.10	2	1.0
Paudel et al. (2019)	*Salmonella*	0.25h	7.2	Cinnamon oil	0.10	1	1.0*
Paudel et al. (2019)	*L. monocytogenes*	0.25h	8.3	Cinnamon oil	0.10	1	0.2*
Paudel et al. (2019)	*L. monocytogenes*	0.25h	8.3	Cinnamon oil	0.25	1	2.1*
Paudel et al. (2019)	*Salmonella*	0.25h	7.2	Cinnamon oil	0.25	1	1.1*
Paudel et al. (2019)	*L. monocytogenes*	0.25h	8.3	Cinnamon oil	0.50	1	2.9*
Paudel et al. (2019)	*Salmonella*	0.25h	7.2	Cinnamon oil	0.50	1	0.8*
Paudel et al. (2019)	*L. monocytogenes (HD)*	0.25h	5.2	Cinnamon oil	0.10	1	-0.5*
Paudel et al. (2019)	*Salmonella (HD)*	0.25h	5.2	Cinnamon oil	0.10	1	-0.5*
Paudel et al. (2019)	*L. monocytogenes (HD)*	0.25h	5.2	Cinnamon oil	0.25	1	-0.2*
Paudel et al. (2019)	*Salmonella (HD)*	0.25h	5.2	Cinnamon oil	0.25	1	-0.2*
Paudel et al. (2019)	*Salmonella (HD)*	0.25h	5.2	Cinnamon oil	0.50	1	-0.3*
Paudel et al. (2019)	*L. monocytogenes (HD)*	0.25h	5.2	Cinnamon oil	0.50	1	-0.2*

* values are from 24h after inoculation, treatment, incubation at unknown temperature, some further declines evident at 72h post-treatment
HD = honeydew

8.2.2.9 X-rays

X-rays		
What is the status as a fruit/vegetable sanitiser?	X-rays are forms of high energy electromagnetic irradiation ('photons') and have been used internationally for decades to disinfect food and medical equipment, and used in a variety of other applications. X-rays are approved to use for specific tropical fruit in Australia to treat for fruit fly.	(FSANZ, 2019) (IIA, 2011)
What is it?	X-rays are forms of ionising radiation. X-rays are produced by using electricity (not radioactive substances) to generate electrons to generate photons. X-rays are generally considered to have a longer wavelength and lower photon energy than gamma rays. X-ray production requires a source of electrons, a means to rapidly accelerate the electrons and, then, a means of rapidly deaccelerating the electrons. This is achieved in commercial systems by using an electron beam accelerator ("e-beam" technology) targeting electrons at a metal plate that deaccelerates the electrons and producing X-rays that are then targeted at the object to be treated.	(Jeong and Jeong, 2018) (Moosekian et al., 2012) (IIA, 2011)
How is it applied?	X-ray treatments have good penetrating power and can be applied to packaged items on a conveyor system. However, they require a specialised building (a 'labyrinth') to prevent the exit of 'stray' X-rays. i.e., to protect workers, and the environment, from ionising irradiation.	(Moosekian et al., 2012)
How does it work?	X-rays cause ionisation and directly damage the DNA of microorganisms, and can disrupt other macromolecules.	(Jeong and Jeong, 2018)
How effective is it?	Reported to be very effective against a range of pathogens and microbiota on produce.	(Moosekian et al., 2012)
What factors influence effectiveness?	Dose, processing speed, target organism.	(Jeong and Jeong, 2018)
Is it approved or regulated?	Currently, only X-ray doses of 1kGy to 150Gy may be used to irradiated select produce for the purpose of pest disinfestation for a phytosanitary objective. Guidance regarding approvals for sanitary purposes would need to be sort from state authorities and FSANZ.	(FSANZ, 2019)
Relative cost?	Investment of > $1,000,000 (est) required. X-rays require a cathode ray generator (vacuum-tube). Extensive shielding is required to contain the X-rays to just the target material.	
What plant or process changes might be required?	Significant changes for the addition of machine-generated X-ray. However, X-rays can be applied after the packaging of rockmelon and could be done in a separate facility.	
Are there environmental considerations?	Relatively environmentally friendly, provided that adequate shielding is provided. Potentially high electrical demand for X-ray generation.	(Moosekian et al., 2012)
Are there OH&S considerations?	Extensive shielding of X-ray units is required to contain the high energy ionizing radiation	(Moosekian et al., 2012)
Other Advantages	No chemical treatment required. Can be conducted after, and in, packaging. Great penetrating power without loss of product quality. Shelf life extension of produce is also observed.	(Ramos et al., 2013; Parish et al., 2003)

	Treatment process can be automated and can be operated at any time. Exposure times for treatments are from 10s of minutes to hours.	
Other Disadvantages	Labelling (with the 'Radura' symbol) is required for irradiated food Current dose limit may not be sufficient to achieve large reductions in *L. monocytogenes* on melons. Consumer acceptance issues	(Ramos et al., 2013; Parish et al., 2003)

Our review identified a single study that investigated the effect of X-rays (i.e., generated by an electron beam system) on the reduction of *L. monocytogenes* and other pathogens on rockmelons. Mahmoud (2012) aimed to investigate the efficacy of X-ray treatments for reducing pathogens on whole rockmelons and to assess the effect on the native microflora, colour and firmness of melons. Rockmelons purchased at retail were spot inoculated with either a three-strain cocktail of *E. coli*, *L. monocytogenes*, or *S. enterica*, or a two-strain cocktail of *Shigella flexneri.* Inoculated melons were then exposed to X-ray treatment doses of 0.1, 0.5, 1.0, 1.5 and 2.0 kGy at a rate of 1.0 kGy/16min, that were generated using an industrial cabinet X-ray irradiator. The results (Table 20) demonstrated that pathogen reductions increased with increasing dose and > 3 $\log_{10}$CFU reductions were achieved with doses of 0.5 kGy for *Shigella* and *Salmonella*, 1.0 kGy for *E. coli*, and 2.0 kGy for *L. monocytogenes*. All pathogens were reduced to undetectable levels when exposed to 2.0kGy. The melon colour or firmness did not differ significantly between untreated or treated (0.1 to 2.0kGy) melons, and mesophilic bacterial counts and yeasts and moulds counts were reduced on treated melons. The shelf life of melons, based on a limit of 5 $\log_{10}$CFU/g yeast and moulds, was extended from 10 days for the control melons to 20 days when treated with 2.0kGy X-ray and stored at 22˚C.

X-ray is a potentially effective means of reducing pathogen numbers on rockmelon surfaces while extending shelf life and retaining quality, and with minimal effect on product quality. However, a long contact time was required in the experiments described but that is, however, a function of the dosing rate of the equipment. In an industrial application it may be possible to deliver these doses at a higher rate to reduce the time required or to process larger volumes at a single time, i.e., X-rays are a penetrating form of radiation and can decontaminate entire pallets of packaged products. However thick shielding of concrete or steel around the treatment chamber is required to prevent workers being exposed to damaging X-rays and to retain the maximum effectiveness for the target products, and this requires considerable capital outlay. Another important limitation to the use of X-ray is that currently only doses of 1kGy to 150Gy may be used to irradiated select

produce for the specific purpose of pest disinfestation for a phytosanitary objective, therefore approval for the specific use of X-ray for removal of pathogens would need to be sort for the treatment of rockmelons (Standard 1.5.3)(FSANZ, 2019). While > 3 log_{10}CFU reductions were seen for other pathogens at this dose, *L. monocytogenes* was only reduced by 2.5 log_{10}CFU. However, further studies are required to demonstrate the reproducibility of these results.

We identified only one study that assessed the efficacy of X-rays to reduce *L. monocytogenes* on rockmelon surfaces. Log_{10}CFU reductions of 0.6 to >4.1 for *L. monocytogenes* on the surface of rockmelons were reported for 0.1 – 2.0kGy doses applied from 1.6 – 32min.

Further research is required and warranted to determine the reproducibility of the results.

Table 20. Studies that assessed the efficacy of X-ray to reduce *L. monocytogenes* and other pathogens on the surface of rockmelons.

Reference	Organism	Attachment time	Initial Log_{10}CFU numbers	Intervention	Dose (kGy)	Contact time (min)	Log_{10}CFU reduction
Mahmoud (2012)	*Shigella*	1h	8.5	X-ray	0.1	1.6	1.8
Mahmoud (2012)	*E. coli*	1h	7.8	X-ray	0.1	1.6	1.7
Mahmoud (2012)	*Salmonella enterica*	1h	8.9	X-ray	0.1	1.6	1.3
Mahmoud (2012)	*L. monocytogenes*	1h	6.6	X-ray	0.1	1.6	0.6
Mahmoud (2012)	*Salmonella enterica*	1h	8.9	X-ray	0.5	8	3.8
Mahmoud (2012)	*Shigella*	1h	8.5	X-ray	0.5	8	3.1
Mahmoud (2012)	*E. coli*	1h	7.8	X-ray	0.5	8	2.7
Mahmoud (2012)	*L. monocytogenes*	1h	6.6	X-ray	0.5	8	1.3
Mahmoud (2012)	*Salmonella enterica*	1h	8.9	X-ray	1.0	16	5.0
Mahmoud (2012)	*E. coli*	1h	7.8	X-ray	1.0	16	4.8
Mahmoud (2012)	*Shigella*	1h	8.5	X-ray	1.0	16	3.7
Mahmoud (2012)	*L. monocytogenes*	1h	6.6	X-ray	1.0	16	2.5
Mahmoud (2012)	*Salmonella enterica*	1h	8.9	X-ray	1.5	24	6.9
Mahmoud (2012)	*Shigella*	1h	8.5	X-ray	1.5	24	6.5
Mahmoud (2012)	*E. coli*	1h	7.8	X-ray	1.5	24	> 5.8
Mahmoud (2012)	*L. monocytogenes*	1h	6.6	X-ray	1.5	24	4.6
Mahmoud (2012)	*Salmonella enterica*	1h	8.9	X-ray	2.0	32	> 6.3
Mahmoud (2012)	*E. coli*	1h	7.8	X-ray	2.0	32	> 5.8
Mahmoud (2012)	*Shigella*	1h	8.5	X-ray	2.0	32	> 5.8
Mahmoud (2012)	*L. monocytogenes*	1h	6.6	X-ray	2.0	32	> 4.1

> indicates results below the limit of detection

8.2.2.10 Ultraviolet-C

Ultraviolet-C light		
What is the status as a fruit/vegetable sanitiser?	Ultraviolet-C light (UV-C) is a commercially available technology used for the disinfection of air, water, fresh produce, conveyor belts and other food contact surfaces.	(Choudhary and Bandla, 2012)
What is it?	Ultraviolet light is a type of electromagnetic wave and is a non-ionising radiation. The different wavelengths of UV light are characterised as UV-A (315 – 400nm), UV-B (280 - 315nm) and UV-C (200 – 280nm). The most common source of UV light is the Sun itself. In commercial systems, however, germicidal UV-C light (254nm) is produced using special lamps (mercury vapour) or now using LED. The mercury vapour lamps produce UV at 254 nm, which is close to the most damaging wavelength for DNA, LED systems operate at slighty longer wavelengths and require longer exposure time for the same efficacy at a given light intensity.	(Choudhary and Bandla, 2012)
How is it applied?	Conveyor and other systems incorporating UV-C lamps.	
How does it work?	UV-C radiation penetrates microbial membranes and directly damages the DNA of target cells by pyrimidine dimerization., leading to cell death.	(Choudhary and Bandla, 2012)
How effective is it?	Active against a range of microorganisms, but less effective against spores. Efficacy differs across produce types, mainly due to the shape of the product and ability for the UV light to reach all surfaces.	(Yoon and Lee, 2018)
What factors influence effectiveness?	Produce type, initial contamination levels, target pathogen, dose. Has poor penetrating properties and efficacy with rockmelons is highly dependent on the surface roughness of the strain.	(Choudhary and Bandla, 2012)
Is it approved or regulated?	UV lights are widely available for use in Australia. Consultation with state authorities is recommended before implementation.	
Relative cost?	Relatively inexpensive.	
What plant or process changes might be required?	Minimal, incorporation of conveyor cabinet. Also, the UV light source requires shielding to protect workers. (Human exposure to UV leads to 'sunburn', also due to cell death (skin cells).	
Are there environmental considerations?	Relatively environmentally friendly. Electricity required, but not unusually high power demand.	
Are there OH&S considerations?	Shielding needed to avoid accidental exposure of workers to UV light. Workplace exposure guidelines are documented.	
Other Advantages	No residues. Non-thermal treatment.	(Choudhary and Bandla, 2012)
Other Disadvantages	Little penetration. Surface application only.	(Choudhary and Bandla, 2012)

From the literature review only one study (Adhikari et al., 2015) was found that investigated the use of ultraviolet light to reduce *L. monocytogenes* on the surface of rockmelons specifically. Adhikari et al. (2015) aimed to determine how different fruit surfaces influence the UV-C inactivation of *L. monocytogenes*. Rockmelons were purchased at retail and 12cm^2 discs were removed, placed in petri dishes, and spot-inoculated with a three strain *L. monocytogenes* cocktail. UV-C light treatments were applied inside a UV-C Emitter^{TM} Table-top System at a wavelength of 254nm at 23°C. The inactivation of *L. monocytogenes* by increasing UV-C doses (kJ/m^2) over 14min was assessed. The greatest reduction was 1 $\log_{10}$CFU, observed at 14min with a dose of 11.9 kJ/m^2 – the maximum tested (Table 21). The inactivation response was not linear, with faster rates in the first two minutes. Inactivation rates for rockmelon were significantly less than for other smoother surfaced fruits tested in the study but, while Adhikari et al (2015) calculated roughness indices for the fruits they considered (including apples, pears, rockmelon, strawberries and raspberries), the correlation between the roughness index and inactivation observed for either *E. coli* or *L. monocytogenes* was poor. They observed only that there was much higher inactivation on apples and pears than on strawberries, raspberries or rockmelons. Curiously, the roughness index for rockmelon in this study was closer to pears than to raspberries (or, in particular, strawberries) yet the inactivation observed on rockmelon was much more similar to raspberries or strawberries than to pears.

Collectively, the results indicate that UV-C alone is not effective at reducing populations of *L. monocytogenes* on rockmelon surfaces, possibly due to the complexity of the surface of netted rockmelons providing shielding/shadowing from the light, i.e., because UV light has very poor penetrating power. Given that 12cm^2 discs of rockmelon were used and were probably oriented directly toward the light source, it might be expected that disinfection of the entire surface of a rockmelon would require UV light coming from many sources/directions, and/or that the rockmelon was being moved constantly so that all surfaces of the melon were exposed to the UV light source for sufficient time.

One study assessed the efficacy of Ultraviolet-C light to reduce *L. monocytogenes* on rockmelon surfaces.

A Log_{10}CFU reduction of 1.0 for *L. monocytogenes* on the surface of rockmelons was reported for 11KJ/m^2 applied for 14min.

We recommend *not* pursuing research into low penetration surface treatments such as UV and other light treatments, alone. However, in hurdle applications (used in combination with other methods) there may be an application for these methods and, in general, research into the application of multiple hurdle/sanitisation technologies is recommended.

Table 21. The efficacy of ultraviolet-C to reduce *L. monocytogenes* and other pathogens on the surface of rockmelons from Adhikari et al. (2015).

Reference	Organism	Attachment time	Initial Log_{10}CFU numbers	Sanitiser	Concentration (kJ/m^2)	Contact time (min)	Log_{10}CFU reduction
Adhikari et al. (2015)	*L. monocytogenes*	1h	6.3	UV-C	11	14	1.0

8.2.2.11 Cold plasma

Cold plasma		
What is the status as a fruit/vegetable sanitiser?	Cold plasma is an emerging technology, and research is being undertaken to evaluate its application and reliability as a fresh produce sanitation method.	(Pignata et al., 2017)
What is it?	Plasma (ionised molecules and electrons as a gas) is considered to be the fourth state of matter. As matter acquires increasing energy it moves from solid to liquid to gas. Plasma is ionised gas where the intramolecular and intra-atomic structures break down, producing a mixture of neutral molecules, electrons, and positive and negative ions. The active particles produced in plasma can react with microorganisms on food. The type of gas ionised determines the reactivity of the plasma. Cold plasma is plasma produced at room temperature and does not cause thermal damage (cooking) to food.	(Niemira, 2012)
How is it applied?	To be determined. There are multiple ways of generating cold plasma. But it is a surface application, similar to UV light.	(Niemira, 2012)
How does it work?	Cold plasma can inactivate microorganisms by damaging DNA, oxidising membranes and intracellular components. Also, the mode of inactivation is dependent on the method of generation.	(Liao et al., 2017)
How effective is it?	Reported to be effective against a range of microorganisms.	(Liao et al., 2017)
What factors influence effectiveness?	Gas type, flow rate, exposure mode, contact time, humidity, target organism. Dependent on the produce type.	(Liao et al., 2017)
Is it approved or regulated?	Will require consultation with state authorities and/or FSANZ regarding potential, reliable, application.	
Relative cost?	Dependent on the method of generation. Likely to be high.	(Niemira, 2012)
What plant or process changes might be required?	"High end" (expensive/sophisticated) technology that will require specialised equipment.	
Are there environmental considerations?	Relatively environmentally friendly. Few environmental outputs.	(Niemira, 2012)
Are there OH&S considerations?	To be determined.	
Other Advantages	Low temperature application (ambient conditions for plasma generation). Has little effect on the quality/attributes of the foods so is potentially ideal for fresh produce.	(Pignata et al., 2017)
Other Disadvantages	In early stages of development for application to commercial food situations. Scale up and optimisation requires significant investment and R&D Complexity of equipment. Antimicrobial modes of action can vary depending on type of cold plasma. Limited penetration ability, i.e. a surface treatment only.	(Pignata et al., 2017)

Our literature search identified only one published report on the use of cold plasma to reduce *L. monocytogenes* on rockmelon surfaces. Jiang et al. (2017) assessed the efficacy of cold plasma activated hydrogen peroxide to reduce *L. innocua* and other bacteria on rockmelon surfaces. They obtained rockmelons from local markets and first washed them with 200ppm chlorine for 2min. $6cm^2$ rind sections were removed and inoculated with strains of *L. innocua*, *E. coli* or *Salmonella*. The rind sections were exposed to 7.8% hydrogen peroxide aerosolized into a treatment chamber using a SteraMistTM BIT^{TM} Activated Ionized Hydrogen Peroxide system. The reason for applying chemical sanitisers when using cold plasma, for which the primary modes of disinfection are due to UV light and reactive chemical products of the cold plasma ionization process are not explained. Separately treated uninoculated melons were used to assess native microbiota changes, and quality parameters of texture, colour, appearance and odour one day after treatment. Treatment reduced *E. coli*, *S.* Typhimurium and *L. innocua* by 4.9, 1.3, and 3.0 $\log_{10}$ CFU/piece, respectively (Table 22). No significant reduction in mesophilic bacteria or yeasts and moulds were achieved for rockmelon rinds: the authors attributed this to the formation of biofilms by native microflora decreasing the efficacy of treatments against them but provided no firm evidence for this suggestion. Firmness and colour were not significantly affected by the treatment.

It should be noted that the treatment of smooth skin tomatoes in the study produced much larger reductions in pathogen numbers with the same treatment. Therefore, the efficacy of this method may be reduced by the inherent roughness of netted rockmelon surfaces, and further optimization may be limited. However, this is an unusual application of cold plasma, and there is scope for other plasma applications to be investigated. But, as the general use of cold plasma is still in the research and development phase, significant capital investment and scale up would likely be required before any commercial application with rockmelon.

One study assessed the efficacy of cold plasma aerolised hydrogen peroxide to reduce *L. monocytogenes* on rockmelon surfaces.

Log_{10}CFU reductions of 3.0 for *L. monocytogenes* on the surface of rockmelons were reported.

Cold plasma is still in the R&D phase and further research is required. The efficacy of this method may be reduced by the inherent roughness of netted rockmelon surfaces, and further optimisation may be limited.

Table 22. Results of Jiang et al (2017) that assessed the efficacy of cold plasma aerosolized hydrogen peroxide to reduce *L. monocytogenes* and other pathogens on the surface of rockmelons.

Reference	Organism	Attachment time	Initial Log_{10}CFU numbers	Sanitiser	Concentration (%)	Contact time (min)	Log_{10}CFU reduction
Jiang et al. (2017)	*E. coli*	2h	5.5	Cold plasma activated hydrogen peroxide	7.8	0.75 + 30min dwell time	4.9
Jiang et al. (2017)	*Listeria innocua*	2h	5.7	Cold plasma activated hydrogen peroxide	7.8	0.75 + 30min dwell time	3.0
Jiang et al. (2017)	*Salmonella*	2h	6.1	Cold plasma activated hydrogen peroxide	7.8	0.75 + 30min dwell time	1.3

8.2.2.12 Lauroyl arginate ethyl

Lauroyl arginate ethyl		
What is the status as a fruit/vegetable sanitiser?	Ethyl lauroyl arginate is authorised for use as a food preservative (E243) and used to prevent microbial growth and food spoilage. Research has investigated its bactericidal application as a wash for fresh produce.	(EFSA FAF Panel, 2019)
What is it?	Ethyl lauroyl arginate is a cationic surfactant. The active ingredient is N-lauroyl-L-arginine ethyl ester hydrochloride.	(FSANZ, 2009)
How is it applied?	In research it has been applied as a wash for produce.	
How does it work?	Ethyl lauroyl arginate works by disrupting the microbial cell membrane, leading to cell death.	(Becerril et al., 2013)
How effective is it?	Is reported to be effective against a broad range of microorganisms.	(Becerril et al., 2013)
What factors influence effectiveness?	Produce type, target organism. Others to be determined.	(Becerril et al., 2013)
Is it approved or regulated?	Requires consultation with state authorities and/or FSANZ regarding *specific* uses. Ethyl lauroyl arginate is an approved food additive.	
Relative cost?	Potentially cost effective.	
What plant or process changes might be required?	Could be incorporated into current sanitising practices.	
Are there environmental considerations?	None identified.	
Are there OH&S considerations?	None identified/reported.	
Other Advantages	Ease of use/application.	
Other Disadvantages	Potential development of antimicrobial resistance	

Fu et al. (2017) aimed to monitor 'biofilm' formation on rockmelon rind with scanning electron cryomicroscopy and to also assess the effect of biofilm formation on the effectiveness of the antimicrobial compound laurel alginate ethyl (LAE) against pathogens on the surfaces of rockmelons. Rockmelons were purchased at retail and rind pieces of 2.25cm^2 were removed and the flesh also removed. The sections were spot inoculated with either a single strain of *L. monocytogenes* or *Salmonella*. 'Biofilm' formation was monitored at 2, 12, 24, and 48h at 22˚C using scanning electron cryomicroscopy. For the treatment of rind sections, at different times following inoculation, pieces were treated for 5min with 10ml of either 200, 400, 800, 1600, 2000 μg/mL LAE solutions at 22˚C. The authors reported ~2 $\log_{10}$CFU increases of both organisms on

rockmelon surfaces stored at 22˚C in 24h. Within 24h both organisms had formed potential 'biofilms' on the rind surfaces. In broth medium the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of LAE for *L. monocytogenes* and *Salmonella* were both determined as 20µg/mL. However, *L. monocytogenes* was more resistant to LAE than *Salmonella* when treating rinds (Figure 5). For *Salmonella*, 2h after inoculation and treated with LAE at 400, and 800µg/mL, reductions were > 2 log_{10}CFU, and at 1600 and 2000ug/mL the pathogens were below the LOD. The efficacy of LAE treatment was reduced for *Salmonella* when applied at 12 and 24h after inoculation, compared to 2h after inoculation. The authors suggested that biofilm formation or attachment time may enhance pathogen resistance to sanitiser. In contrast, no concentration of LAE at any time point was able to reduce *L. monocytogenes* by >1 log_{10}CFU and, contrary to the author claims, no evidence of biofilm formation providing enhanced resistance to sanitisers was evident. Given that the MIC and MBC for both pathogens were similar in broth, *L. monocytogenes* was assumed to attach more efficiently and be more resistant to LAE than *Salmonella* on rockmelon rinds. However, LAE was not effective at removing *L. monocytogenes* at any concentration. Thus, to determine the possible effect of biofilm formation on the efficacy of sanitisers, use of a sanitiser known to be effective against *L. monocytogenes* on rockmelon rind would prove interesting. Furthermore, this study only used single strains of challenge organisms and was not conducted on whole melons. The potential for biofilm formation to reduce sanitiser effectiveness is relevant to the industry as it is not known at what point *L. monocytogenes* contamination may occur on rockmelons. As biofilm formation may occur in the field, any future studies of sanitisers should be designed to demonstrate their efficacy against biofilms.

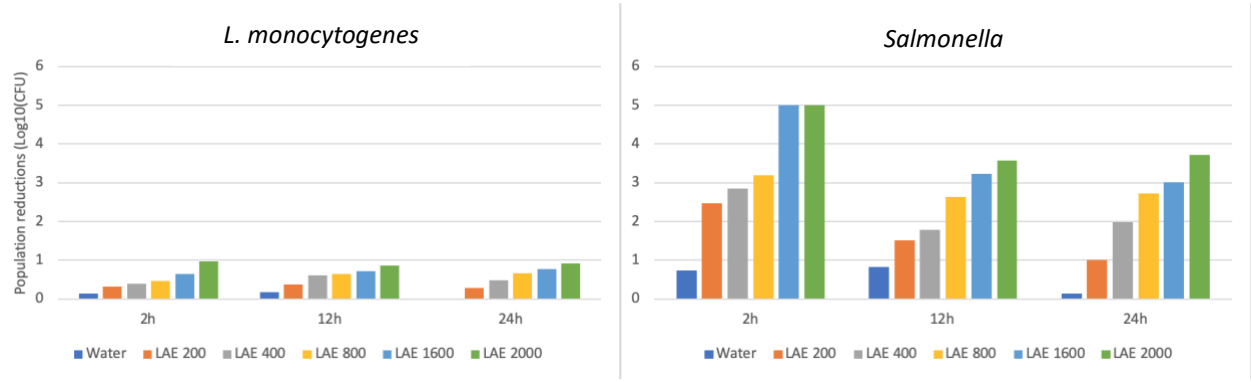

Figure 5. Log_{10}CFU reductions of *L. monocytogenes* and *Salmonella* inoculated onto rockmelon rind then stored for 2, 12, and 24h before treatment with either water or laurel alginate ethyl (LAE) at different concentrations.

8.2.2.13 Electrolysed water

Electrolysed water		
What is the status as a fruit/vegetable sanitiser?	Electrolysed water is a commercially available technology used to sanitise fresh produce, and surfaces and has applications in areas such as agriculture, food processing and medicine.	(Rahman et al., 2016)
What is it?	Electrolysed water is generated using normal water containing sodium chloride (NaCl) exposed to an electric current. In an electrolysed water generator the anode and cathode can be separated by a membrane and electrolysed acidic solution (pH 2 - 3) is produced at the anode and electrolysed basic solution (pH 10 - 13) is produced at the cathode. This is due to the dissociation of NaCl and water (H_2O) into positively and negatively charged ions that then combine to form hypochlorous acid (HOCl), hypochlorite ion (−OCl), hydrochloric acid (HCl), oxygen gas (O_2), and chlorine gas (Cl_2) at the anode or sodium hydroxide (NaOH) and hydrogen gas at the cathode. Near neutral electrolysed (pH 6 – 7) water can be produced in generators without a membrane between the anode and cathode or mixing acidic or basic electrolysed water.	(Rahman et al., 2016)
How is it applied?	As a wash.	
How does it work?	The hypochlorite ion damages the outer microbial membrane. Hypochlorous acid can diffuse through the cell membrane damaging both the membrane and vital cell components.	(Rahman et al., 2016)
How effective is it?	Active against a wide array of microorganisms. Differing results for different produce.	
What factors influence effectiveness?	Produce type, pH, water type, organic matter, water temperature, storage conditions, current, flow rate, electrolyte, and electrode materials.	(Rahman et al., 2016) (Yoon and Lee, 2018)
Is it approved or regulated?	Commercial electrolysed water methods are available in Australia. Consultation with state authorities is recommended before implementation.	
Relative cost?	High initial cost for commercial generator. Reported to be cost effective. The electricity, chemicals (NaCl) and water are main operational costs but are all relatively inexpensive.	(Rahman et al., 2016)
What plant or process changes might be required?	Commercial generator would need to be installed.	
Are there environmental considerations?	Relatively environmentally friendly, similar considerations as for use of hypochlorite/bleach (*see* Section 8.2.1.1).	(Rahman et al., 2016)
Are there OH&S considerations?	Chlorine gas generated if production of acidic electrolysed water pH < 5, chlorine gas is highly oxidising and can harm workers who inhale it. Phytotoxicity, irritation of hands, and corrosion of equipment. Safe Work Australia exposure standards are documented for chlorine	(Rahman et al., 2016) (Ramos et al., 2013)

Other Advantages	Neutralizes harmful substances such as cyanides and ammonium On site generation (so that large volumes of hypochlorite solutions do not need to be shipped to the processing plant).	(Rahman et al., 2016) (Ramos et al., 2013)
Other Disadvantages	Can reduce quality of fresh-cut vegetables Antimicrobial efficacy of electrolysed water can diminish quickly if not properly stored and managed.	

Our literature review identified a single published report that assessed the ability of electrolysed water to reduce *L. monocytogenes* and other pathogen populations on the surface of rockmelons. Singh et al. (2018), introduced in Section 8.2.1.1, compared the efficacy of a number of sanitisers including acidified and near-neutral electrolysed water. The acidified and near-neutral electrolysed water were prepared the day before the experiment by electrolysing a dilute salt solution (NaCl: 0.03%) using an EAU Technologies electrolysed water generator and diluting to adjust to 100ppm free chlorine. The pH of acidified and near neutral electrolysed water was adjusted to 2.5 and 6.2 respectively. The same methods as for chlorine were used (Section 8.2.1.1) to treat melons for 5min and, notably, the temperature of sanitisers was 4˚C. The results (Table 23), showed that *L. monocytogenes* was the most resistant to treatment with both acidic and near neutral electrolysed water and demonstrated log_{10}CFU reductions of 1.7 and 2.1 respectively. In this study, neither acidic nor near neutral electrolysed water were the most effective sanitiser tested for either pathogen. However, it is known that its effectiveness increases with increasing temperature, which is generally true for chemical sanitisers. Therefore, there is insufficient evidence to determine the efficacy of electrolysed water to decontaminate rockmelon surfaces in comparison to other sanitisers/technologies.

We identified only one study that assessed the efficacy of electrolysed water to reduce *L. monocytogenes* on rockmelon surfaces.

Log_{10}CFU reductions of 1.7 and 2.1 for *L. monocytogenes* on the surface of rockmelons were reported for Acidic and near neutral electrolysed water applied at 4˚C for 5min.

There is insufficient evidence to determine the efficacy of electrolysed water to decontaminate rockmelon surfaces in comparison to other sanitisers/technologies.

Table 23. Overview of the results of the only published study (Singh et al., 2017) that assessed the efficacy of electrolyzed water (EO) to reduce *L. monocytogenes* and other pathogens on the surface of rockmelons.

Reference	Organism	Attachment time	Initial Log_{10}CFU numbers	Sanitiser	Free chlorine concentration (%)	Contact time (min)	Log_{10}CFU reduction
Singh et al. (2018)	*S.* Typhimurium	Overnight	6.6	Acidic EO	100	5	2.3
Singh et al. (2018)	*L. monocytogenes*	Overnight	7.6	Acidic EO	100	5	1.7
Singh et al. (2018)	*S.* Typhimurium	Overnight	6.6	Near neutral EO	100	5	3.7
Singh et al. (2018)	*L. monocytogenes*	Overnight	7.6	Near neutral EO	100	5	2.1

8.2.3 Summary of the post-processing sanitisation methods that have produced > 3 log reductions in *L. monocytogenes* or other pathogens on the surface of melons

There is a desire in the rockmelon industry to identify interventions and sanitisers that can produce a greater than 3 log_{10}CFU reduction in *L. monocytogenes* on the surface of rockmelons. As such, in this section we have summarised the information for the sanitisers presented in the previous Section and only reported the interventions that produced a greater than 3 log_{10}CFU reduction in pathogens, in order of decreasing efficacy. Summary tables for *L. monocytogenes* , *E. coli* , and *Salmonella* inactivation are presented below.

The results presented below further demonstrate that the sanitisers applied under experimental conditions at concentrations and contact times currently used by the Australian industry (*i.e.,* < 2min) do not produce > 3 log_{10}CFU reductions. We also noted a general trend across most studies of *L. monocytogenes* being more resistant to the sanitisers tested than either *E. coli o*r *Salmonella*. However, this was not true for *all* studies and requires further investigation.

Where the '>' symbol appears in front of a log_{10}CFU reduction value in the tables, this indicates a result where the pathogen was reduced to below the limit of detection, *i.e.*, that it is possible that the actual inactivation was larger than what is reported (This limitation is further discussed in Section 11).

There are a number of sanitisers that have been assessed under laboratory conditions that produced > 3 log_{10}CFU reductions in *L. monocytogenes*. However, due to limited research, cost, practicality, and other considerations, not all of these will be relevant for the Australian industry at this time. Determination of cost/benefit for these proposed treatments was beyond the scope of this review.

Based on the results of the review we have recommended that further research into methods that have demonstrated relatively high effectiveness against *L. monocytogenes* such as ozone, X-ray, octenidine dihydrochloride, hot water, superheated steam, and dry steam including determination of their costs versus benefits, may be relevant.

Table 24. Studies that reported interventions that produced a greater than 3 log_{10}CFU reduction in *L. monocytogenes* on the surface of melons in order of decreasing efficacy.

Reference	Attachment time (h)	Initial numbers Log_{10}CFU	Sanitiser and treatment conditions	Contact time (min)	Log_{10}CFU reduction
Kwon et al. (2019)	1	NR	Lactic acid 2% 2.12 pH 1min + superheated steam 200˚C 20s (Section 8.2.2.3)	1.34	> 5.4
Kwon et al. (2019)	1	NR	Lactic acid 2% 2.12 pH 1min + superheated steam 200˚C 20s (Section 8.2.2.3)	1.34	> 5.4
Hwang et al. (2017)	20min	NR	Chlorine dioxide. Sequential application by immersion in 35ml of sodium chlorite (1.6 %) and 35ml of hydrochloric acid (6mM, i.e., 0.05% solution of 12 M HCl) (Section 8.2.2.1)	60	5.2
Upadhyay et al. (2016)	2	5.6	Octenidine dihydrochloride 0.1% + Chitosan 2% coating (Section 8.2.2.7)	1440	>5.0
Kwon et al. (2018)	1	6.0	Superheated steam 200˚C (Section 8.2.2.3)	0.5	>5.0
Rodgers et al. (2004)	24	~6.0	Chlorine 100ppm (Section 8.2.1.1)	5.0	>5.0
Rodgers et al. (2004)	24	~6.0	Chlorine 200ppm (Section 8.2.1.1)	5.0	>5.0
Rodgers et al. (2004)	24	~6.0	Chlorine dioxide (aqueous) 3ppm (Section 8.2.1.2)	5.0	>5.0
Rodgers et al. (2004)	24	~6.0	Chlorine dioxide (aqueous) 5ppm (Section 8.2.1.2)	5.0	>5.0
Rodgers et al. (2004)	24	~6.0	Chlorine dioxide (aqueous) 5ppm (Section 8.2.1.2)	5.0	>5.0
Rodgers et al. (2004)	24	~6.0	Ozone (aqueous) 3ppm (Section 8.2.1.4)	5.0	>5.0
Kwon et al. (2019)	1	NR	Lactic acid 2% 2.12 pH 1min + superheated steam 150˚C 20s (Section 8.2.2.3)	1.34	4.7
Mahmoud (2012)	1	6.6	X-ray 1.5 kGy (Section 8.2.2.9)	32	> 4.6
Zhang et al. (2015)	NR	5.9	Cinnamon bark oil 2% + Alginate 1.0% coating (Section 8.2.2.7)	1440	> 4.6
Zhang et al. (2015)	NR	5.9	Cinnamon bark oil 2 % + Soybean oil 0.5% + Alginate 1.0% coating (Section 8.2.2.7)	1440	> 4.6
Singh et al. (2018)	Overnight	7.6	Peracetic acid 100ppm (Section 8.2.1.3)	5	4.5
Kwon et al. (2019)	1	NR	Lactic acid 2% 2.12 pH 1min + superheated steam 150˚C 20s (Section 8.2.2.3)	1.34	4.1
Kwon et al. (2019)	1	NR	Lactic acid 2% 2.12 pH 1min + saturated steam 100˚C 20s (Section 8.2.2.3)	1.34	4.1
Ma et al. (2016)	6	5.7	Lauric arginate 0.1% + ethylenediaminetetraacetic acid 0.1% + cinnamon oil 1.0% + Chitosan 1.0% coating (Section 8.2.2.7)	1440	> 4.1

Upadhyay et al. (2016)	2	5.6	Octenidine dihydrochloride 0.2% + Chitosan 2% coating (Section 8.2.2.7)	1440	> 4.1
Mahmoud (2012)	1	6.6	X-ray 2.0 kGy (Section 8.2.2.9)	40	4.1
Kozempel et al., (2002)	1	5.6	Vacuum/Steam/Vacuum (Section 8.2.2.3)	0.017	4.1
Ukuku & Fett (2002)	24	4.1	Chlorine (hypochlorite) 1000ppm (Section 8.2.1.1)	2	> 4.0
Ukuku & Fett (2002)	24	4.1	Hydrogen peroxide 5% (Section 8.2.2.2)	2	> 4.0
Ma et al. (2016)	6	5.7	Chitosan 1% coating (Section 8.2.2.7)	1440	4.0
Forney et al. (2015)	18 – 20	5.0	Vapour heat 84˚C (Section 8.2.2.3)	4	4.0
Kwon et al. (2018)	1	6.0	Superheated steam 150˚C (Section 8.2.2.3)	0.5	4.0
Bezanson et al. (2018)	18 - 20	5.3	Aerated steam 85˚C (Section 8.2.2.3)	4	3.9
Kwon et al. (2019)	1		Lactic acid 2% 2.12 pH 1min + saturated steam 100˚C 20s (Section 8.2.2.3)	1.34	3.8
Ukuku et al. (2016)	24	4.1	Wet steam 68˚C (Section 8.2.2.3)	3	3.8
Kwon et al. (2018)	1	6.0	Saturated steam 100˚C (Section 8.2.2.3)	0.5	3.6
Upadhyay et al. (2016)	2	5.6	Octenidine dihydrochloride 0.1% wash (Section 8.2.2.6)	5	> 3.6
Upadhyay et al. (2016)	2	5.6	Octenidine dihydrochloride 0.2% wash (Section 8.2.2.6)	5	> 3.6
Kozempel et al., (2002)	1	5.4	Vacuum/Steam/Vacuum (Section 8.2.2.3)	0.0083	3.5
Ma et al. (2016)	6	5.7	Lauric arginate 0.1% + ethylenediaminetetraacetic acid 0.1% + Chitosan 1.0% coating (Section 8.2.2.7)	1440	3.4
Ukuku et al. (2016)	2	3.6	3% Hydrogen peroxide at 80°C (Section 8.2.2.3)	5	> 3.3
Ukuku et al. (2016)	2	3.6	Water at 80°C (Section 8.2.2.3)	5	> 3.3
Ukuku et al. (2016)	24	4.1	Wet steam 68˚C (Section 8.2.2.3)	3	3.3
Trinetta et al. (2013)	1	6.1	Chlorine dioxide gas 10mg/L (Section 8.2.2.1)	3	3.3
Ma et al. (2016)	6	5.7	Lauric arginate 0.1% + ethylenediaminetetraacetic acid 0.1% + cinnamon oil 0.5% + Chitosan 1.0% coating (Section 8.2.2.7)	1440	3.1

Table 25. Studies that reported interventions that produced a greater than 3 log_{10}CFU reduction in *E. coli* on the surface of melons in order of decreasing efficacy.

Reference	Attachment time (h)	Initial numbers Log_{10}CFU	Sanitiser and treatment conditions	Contact time (min)	Log_{10}CFU reduction
Mahmoud (2012)	1	7.8	X-ray 1.5 kGy (Section 8.2.2.9)	32	> 5.8
Mahmoud (2012)	1	7.8	X-ray 2.0 kGy (Section 8.2.2.9)	40	> 5.8
Kwon et al. (2019)	1	6.5	Lactic acid 2% 2.12 pH 1min + Superheated steam 200˚C 20s (Section 8.2.2.3)	1.34	5.5
Kwon et al. (2018)	1	6.7	Superheated steam 200˚C (Section 8.2.2.3)	0.5	> 5.3
Kwon et al. (2019)	1	6.5	Lactic acid 2% 2.12 pH 1min + Superheated steam 200˚C 20s (Section 8.2.2.3)	1.34	5.2
Jiang et al. (2017)	2	5.5	Cold plasma activated hydrogen peroxide (7.8%) (Section 8.2.2.11)	0.75 + 30min dwell time	4.9
Ukuku et al. (2016)	2	5.1	3% Hydrogen peroxide at 80°C (Section 8.2.2.3)	5	> 4.8
Ukuku et al. (2016)	2	5.1	Water at 80°C (Section 8.2.2.3)	5	> 4.8
Mahmoud (2012)	1	7.8	X-ray 1.0 kGy (Section 8.2.2.9)	16	4.8
Kwon et al. (2019)	1	6.5	Lactic acid 2% 2.12 pH 1min + Superheated steam 150˚C 20s (Section 8.2.2.3)	1.34	4.6
Upadhyay et al. (2016)	2	5.6	Octenidine dihydrochloride 0.1% wash (Section 8.2.2.6)	5	4.5
Ukuku et al. (2016)	24	4.8	Wet steam 68˚C (Section 8.2.2.3)	3	4.4
Ukuku et al. (2005)	1	5.3	HPLNC; hydrogen peroxide (1%) in combination with nisin (25 ug/ml), sodium lactate (1%), and citric acid (0.5%) (Section 8.2.2.2)	5	4.4
Kwon et al. (2018)	1	6.7	Superheated steam 150˚C (Section 8.2.2.3)	0.5	4.3
Kwon et al. (2019)	1	6.5	Lactic acid 2% 2.12 pH 1min + Superheated steam 150˚C 20s (Section 8.2.2.3)	1.34	4.3
Ukuku et al. (2016)	2	5.1	3% Hydrogen peroxide at 20°C (Section 8.2.2.3)	5	4.1
Kwon et al. (2019)	1	6.5	Lactic acid 2% 2.12 pH 1min + saturated steam 100˚C 20s (Section 8.2.2.3)	1.34	3.8
Upadhyay et al. (2016)	2	5.6	Octenidine dihydrochloride 0.2% wash (Section 8.2.2.6)	5	> 3.6
Kwon et al. (2019)	1	6.5	Lactic acid 2% 2.12 pH 1min + saturated steam 100˚C 20s (Section 8.2.2.3)	1.34	3.5
Ukuku et al. (2016)	24	4.8	Wet steam 68˚C (Section 8.2.2.3)	3	3.4
Ma et al. (2016)	6	5.0	Lauric arginate 0.1% + ethylenediaminetetraacetic acid 0.1% + chitosan 1.0% coating (Section 8.2.2.7)	1440	3.2

Table 26. Studies that reported interventions that produced a greater than 3 log reduction in *Salmonella* on the surface of melons in order of decreasing efficacy.

Reference	Attachment time (h)	Initial numbers Log_{10}CFU	Sanitiser and treatment conditions	Contact time (min)	Log_{10}CFU reduction
Mahmoud (2012)	1	8.9	X-ray 1.5 kGy (Section 8.2.2.9)	32	> 6.9
Mahmoud (2012)	1	8.9	X-ray 2.0 kGy (Section 8.2.2.9)	40	6.3
Kwon et al. (2019)	1	NR	Lactic acid 2% 2.12 pH 1min + superheated steam 200˚C 20s (Section 8.2.2.3)	1.34	5.3
Hwang et al. (2017)	20min	5.6	Chlorine dioxide. Sequential application by immersion in 35ml of sodium chlorite (1.6 %) and 35ml of hydrochloric acid (6mM, i.e., 0.05% solution of 12 M HCl) (Section 8.2.2.1)	60	5.1
Kwon et al. (2018)	1	6.0	Superheated steam 200˚C (Section 8.2.2.3)	0.5	> 5.0
Fu et al. (2017)	2	6.0	Laurel alginate ethyl 1600μg/mL (Section 8.2.2.12)	5	> 5.0
Fu et al. (2017)	2	6.0	Laurel alginate ethyl 2000μg/mL (Section 8.2.2.12)	5	> 5.0
Mahmoud (2012)	1	8.9	X-ray 1.0 kGy (Section 8.2.2.9)	16	5.0
Kwon et al. (2019)	1	NR	Lactic acid 2% 2.12 pH 1min + superheated steam 200˚C 20s (Section 8.2.2.3)	1.34	> 4.9
Zhang et al. (2016)	2.5	6.9	Thyme oil emulsion 0.2% (Section 8.2.2.8)	2	4.7
Kwon et al. (2018)	1	6.0	Superheated steam 150˚C (Section 8.2.2.3)	0.5	4.7
Ukuku et al. (2016)	2	4.8	3% Hydrogen peroxide at 80°C (Section 8.2.2.3)	5	> 4.5
Ukuku et al. (2016)	2	4.8	Water at 80°C (Section 8.2.2.3)	5	> 4.5
Singh et al. (2018)	Overnight	6.6	Peracetic acid 100ppm (Section 8.2.1.3)	5	4.5
Upadhyay et al. (2016)	2	5.6	Octenidine dihydrochloride 0.1% wash (Section 8.2.2.6)	5	4.5
Singh et al. (2018)	Overnight	6.6	Peracetic acid 85ppm (Section 8.2.1.3)	5	4.2
Kwon et al. (2019)	1	NR	Lactic acid 2% 2.12 pH 1min + superheated 150˚C 20s (Section 8.2.2.3)	1.34	4.1
Ukuku et al. (2016)	24	4.5	Wet steam 68˚C (Section 8.2.2.3)	3	4.1

Kwon et al. (2019)	1	NR	Lactic acid 2% 2.12 pH 1min + superheated 150˚C 20s (Section 8.2.2.3)	1.34	4.0
Trinetta et al. (2013)	1	5.9	Chlorine dioxide gas 10mg/L (Section 8.2.2.1)	3	4.0
Kwon et al. (2018)	1	6.0	Saturated steam 100˚C (Section 8.2.2.3)	0.5	4.0
Upadhyay et al. (2016)	2	5.6	Octenidine dihydrochloride 0.1% + Chitosan 2% coating (Section 8.2.2.7)	1440	> 3.9
Upadhyay et al. (2016)	2	5.6	Octenidine dihydrochloride 0.2% + Chitosan 2% coating (Section 8.2.2.7)	1440	> 3.9
Singh et al. (2018)	Overnight	6.6	Chlorine (hypochlorite) 100ppm (Section 8.2.1.1)	5	3.8
Mahmoud (2012)	1	8.9	X-ray 0.5 kGy (Section 8.2.2.9)	8	3.8
Fu et al. (2017)	48	8.1	Laurel alginate ethyl 2000µg/mL (Section 8.2.2.12)	5	3.7
Singh et al. (2018)	Overnight	7.9	Lactic acid 2%	5	3.7
Singh et al. (2018)	Overnight	6.6	Near neutral electrolysed water (Section 8.2.2.13)	5	3.7
Singh et al. (2018)	Overnight	7.9	Peracetic acid 45ppm (Section 8.2.1.3)	5	3.6
Fu et al. (2017)	24	7.7	Laurel alginate ethyl 2000µg/mL (Section 8.2.2.12)	5	3.6
Kwon et al. (2019)	1	NR	Lactic acid 2% 2.12 pH 1min + saturated steam 100˚C 20s (Section 8.2.2.3)	1.34	3.5
Upadhyay et al. (2016)	2	5.6	Octenidine dihydrochloride 0.2% wash (Section 8.2.2.6)	5	> 3.5
Kwon et al. (2019)	1	NR	Lactic acid 2% 2.12 pH 1min + saturated steam 100˚C 20s (Section 8.2.2.3)	1.34	3.3
Zhang et al. (2015)	NR	4.6	Cinnamon bark oil 2% + Alginate 1.0% coating (Section 8.2.2.7)	1440	> 3.3
Zhang et al. (2015)	NR	4.6	Cinnamon bark oil 2 % + Soybean oil 0.5% + Alginate 1.0% coating (Section 8.2.2.7)	1440	> 3.3
Fu et al. (2017)	24	7.7	Laurel alginate ethyl 1600µg/mL (Section 8.2.2.12)	5	3.2
Fu et al. (2017)	2	6.0	Laurel alginate ethyl 800µg/mL (Section 8.2.2.12)	5	3.2

8.2.4 Other post-harvest interventions for the reduction of *L. monocytogenes* on the surface of melons

There are limited studies that specifically assess the reduction of *L. monocytogenes* on the surface of whole melons via additional post-harvest interventions during other aspects of melon processing following or before the primary sanitisation of melons.

8.2.4.1 Forced air cooling enhancement with aerosolised sanitisers:

Wu (2014) undertook initial research to assess the efficacy of aerosolised sanitisers during forced air cooling to reduce *L. monocytogenes* and *Salmonella* on the rind of melons. Rockmelons were purchased at retail and the rind removed and cut into 1g sections. The rind sections were spot inoculated with a single strain of *L. monocytogenes* and *Salmonella* and allowed to dry for 2h before exposure to sanitisers. Inoculated rind pieces were placed inside and on top of a packing box stacked on top of another box that held the uninoculated rind pieces in a laboratory-scale forced air cooling chamber equipped with an ultrasonic mister. 8L of the following antimicrobials were aerosolised in the chamber with the ultrasonic mister: Chs = chlorinated solution at 200 ppm +100 ppm SDS, pH adjusted to pH=6.5; S = 100 ppm SDS; TcinScar = 400 ppm thymol+ 400 ppm trans-cinnmaldehyde+100 ppm SDS+400ppm carcacrol; ToCS = 4 mg/mL thyme oil +2.5% citric acid+1000 ppm SDS; ToChS = 4 mg/mL thyme oil + 500 ppm chlorine+1000 ppm SDS. Rind was exposed at 4˚C for 180min. The results of that study are reproduced in Figure 6, which shows that the reductions do not exceed 2 $\log_{10}$CFU and, as is evident in Figure 6 and that the variability of results is high in many treatments. This is possibly due to the inconsistent entry of the aerosolised sanitiser into the boxes, however, even the rind samples outside of the boxes display a high degree of variability in the $\log_{10}$CFU reductions of both pathogens. As discussed by the authors, the application of this technology could help to further decrease the risk from *L. monocytogenes*, however, further research is required to understand the source of the variability in these experiments, optimise the technology and choice of sanitisers, and to validate results at an industry-relevant scale.

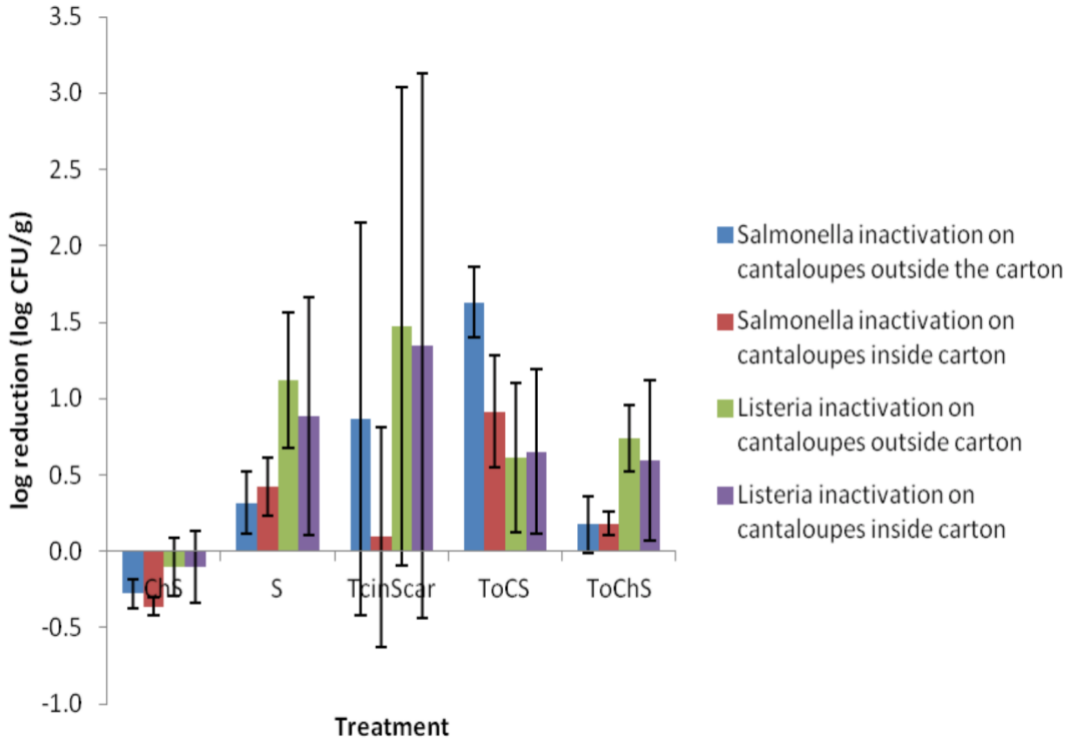

Figure 6. Reproduced from Wu (2014): The impacts of antimicrobial formulations and exposure locations on *Salmonella and L. monocytogenes* inactivation during forced air (containing aerosolised sanitisers) cooling of cantaloupes. (Data are average and standard deviation of 3 independent trials, treatment duration 180min). Chs = chlorinated solution at 200 ppm +100 ppm SDS, pH adjusted to pH=6.5; S, 100 ppm SDS; TcinScar = 400 ppm thymol+ 400 ppm trans-cinnmaldehyde+100 ppm SDS+400ppm carcacrol; ToCS = 4 mg/mL thyme oil +2.5% citric acid+1000 ppm SDS; ToChS = 4 mg/mL thyme oil + 500 ppm chlorine+1000 ppm SDS.

8.2.4.2 Blue light emitting diodes (LEDs)

Josewin et al. (2018) assessed the efficacy of blue light emitting diodes (LEDs) of 405nm and 460nm wavelengths with and without Na-Chl (sodium chlorophyllin copper solution) against *L. monocytogenes* and *Salmonella* on rockmelon rinds. The rockmelons were purchased at retail and 4cm rind sections were separated from the flesh and autoclaved at 121˚C for 15min to remove background microbiota. Rind sections were spot inoculated with either a three-strain cocktail of *L. monocytogenes* or *Salmonella*. Inoculated rinds were immersed in Na-Chl, and following drying, were illuminated under 405 (7 ± 2mW/cm2) or 460nm LEDs (31 ± 3mW/cm2) for 48h at 4 and 20°C. The results demonstrated that the inactivation curves were non-linear. The time for a 1 log_{10}CFU reduction of the pathogen on the rockmelon rind as calculated from the Weibull model for each treatment are presented in Table 27 and are, notably, quite long.

Table 27. Results from Josewin et al. (2018) assessing the efficacy of blue light emitting diodes on inactivation of *L. monocytogenes* and *Salmonella* spp. on rockmelon surfaces.

	Time to yield a 1 log_{10}CFU reduction of the pathogen on the *rockmelon* rind. (h)			
	405 nm	405 nn + 100 pM Na-Chl	460 nm	460 nm + 100 pM Na-Chl
4 °C				
L. monocytogenes	3.48	2.40	6.10	4.70
Salmonella spp.	12.26	11.90	37.69	36.30
20 °C				
L. monocytogenes	2.12	1.50	12.44	6.25

The use of this technology during transport, as posited by the authors, is interesting. However, there are barriers in terms of scale-up to meet industry needs. The time required at 4˚C at 405nm for more than a 1 log_{10}CFU decrease is greater than 3h for both pathogens. Another issue is the ability to use this on the surface of a whole rockmelon and provide consistent contact time and intensity. While the use of this method in post-processing is unlikely, it could be potentially used as an additional safety precaution but may have more of an application at the retail level when displaying fruit in single layers.

The potential for additional risk reduction using combinations of sanitisation methods post-harvest, in addition to primary sanitisation of whole melons, is understudied.

We recommend further research into hurdle technology and combination processes.

8.3 Other potential interventions not identified in the review

In this review we considered and reviewed only authoritative reports that describe the effectiveness of interventions to reduce specifically *L. monocytogenes* on rockmelons. However, there are other technologies that may be feasible and useful, but which have not been explicitly tested for their efficacy against *L. monocytogenes* on rockmelon surfaces. Accordingly, in this section we discuss technologies that may be of interest to the Australian melon industry but that may have not yet had their efficacy rigorously assessed. This section, however, is *not* a comprehensive review of all available intervention technologies.

8.3.1 Gamma irradiation

Gamma irradiation is a form of high energy ionising radiation, generated by radioactive metal isotopes such as Cobalt 60 or Cesium 137. It is widely considered as safe for disinfection of produce, leaves no residues, and does not reduce product quality. While gamma irradiation has been used to disinfect fresh produce and is approved for use on produce in Australia (FSANZ, 2019), and other nations, we found no studies specifically on the effect of gamma irradiation on *L. monocytogenes* on, or in, rockmelons. In Australia, to date, gamma irradiation has mainly been used for pest disinfestation or sterilisation of single-use medical equipment such as syringes, dressings, and petri-plates. It is, however, an expensive technology.

8.3.2 Biological treatments

The use of biocontrol methods is increasing in research and breadth of application. Biocontrol methods can include the use of bacteriophages, endolysins, competitive bacterial species, and bacteriocins. Gray et al. (2018) reviews novel biocontrol methods for *L. monocytogenes* biofilms in production facilities.

8.3.3 Novel and emerging technologies integrated into industrial systems for fruit surface sanitation

As mentioned above (Section 8.2), proprietary fruit sanitation systems are commercially available but evidence for their efficacy is often limited to the manufacturer's data and promotional literature, rather than the published, refereed, literature.

The effectiveness of a commercial sanitisation method is optimised by the design and controls of the equipment, *e.g.,* the safety of the chemicals or treatment is usually optimised by the

automation of the delivery system eliminating manual processes and operator exposure. Additionally, *validation* of effective operation is often incorporated into the automation so that records are made for food safety verification purposes (*e.g.*, HACCP plans).

Consequently, in this section we discuss a range of propriety systems that could be used for surface sanitation in the Australian rockmelon industry. Some of the emerging technologies, *e.g.,* based around ozone or plasma technologies (*see* Section 8.2.1.4 and 8.2.1.11) are still being developed for commercial implementation. In this Section, no endorsement of any of the systems described is given or implied.

8.3.3.1 Continuous conveyer belt sanitation

In situations where processes are stopped for periodic sanitation, continuous belt sanitation systems are not needed. However, if needed, there are systems available to remove microbial contamination that could affect the safety of the produce. These systems are usually based on steam but are not needed for fruit processing where wet oxidative sanitation *(i.e.,* involving chemical sanitisers) systems can be applied.

8.3.3.2 Continuous conveyer fruit sanitation

As discussed in Section 8.2, systems that treat rockmelons on a conveyer immediately prior to packing are actively used in Australia. Mostly they are based on the use of chemical sanitisers (hypochlorite, chlorine dioxide, aqueous ozone) or combinations of treatments. The fruit is exposed to the active agents in a controlled manner to ensure that all surfaces are treated. However, other systems are available. These are discussed below.

8.3.3.3 Photohydronization

Photohydronization (https://www.rgf.com/phi) is a propriety approach for tunnel applications that uses both UV light and a metallic catalyst to generate a range of oxidation products that can disinfect fruit surfaces.

8.3.3.4 Pulsed Light

Pulsed light is another treatment that can be integrated into fruit treatment (disinfection) tunnels. Pulsed light involves short and intense pulses of a broad spectrum of light (200–1100 nm) ranging from UV to near infrared although the disinfection is mostly associated with the UV spectrum.

Pulsed light has advantages in that the high intensity minimises the energy costs and heating damage that can occur if the intense light is applied continuously.

8.3.3.5 Plasma-generated oxidising systems

Plasma generated ozone and plasma activated water ('PAW') are being developed for pathogen control in fruits and vegetables (*see* also Section 8.2.2.11). The technologies produce reactive oxygen species that can inactivate microbes including *L. monocytogenes*. Ozone is generated by electrical discharge and can be applied as a gas or dissolved in water.

PAW is also produced from electrical discharges but is thought to generate a wider range of reactive oxygen species. However, we could find no commercial systems for fruit disinfection.

8.3.3.6 Electrolytically generated oxidising systems

Water-based electrolytic systems (based on slightly saline solutions, *see* Section 8.2.2.13) can also generate reactive oxygen species for sanitation. Unlike PAW they are a mature, existing, technology, and available from various manufacturers, *e.g.,*

Unipolar http://unipolarwater.com/applications

Envirolyte http://www.envirolyte.com/products.html

8.3.4 Antimicrobial gas generator systems for postharvest shipment and storage

8.3.4.1 Sulfur dioxide and hydrogen peroxide antimicrobial vapour dispensing sachets

Environmentally activated systems for dispensing antimicrobial vapours are now being used for postharvest shelf life extension of fruits and vegetables by treatment during shipment and storage. The systems are activated by humidity to slowly release the active gas at low concentrations. A primary target for shelf life extension is the removal of ethylene by oxidation but does not specifically relate to food safety. However, sulphur compounds and hydrogen peroxide (*see* Section 8.2.2.2) are broad-spectrum disinfectants. Thus, through their generic mode of action, they could also inactivate surface pathogens and suppress microbial growth but require closed containment of the gas with packaged produce to be effective. This is currently unlikely to be workable with Australian rockmelon production, processing and distributions systems.

Examples are Coolsan Chillsafe (https://coolsan.com.au/) and Biopac (http://www.biopac.com.au/). Neither system has been validated for rockmelons but the application to extend the shelf life through ethylene control could have a cross-over effect on microbial contamination.

Our expert team identified potential interventions that may be of interest to the industry but that have not yet had their efficacy tested with melons for inactivation of *L. monocytogenes.*

However, before adoption by packhouses, we recommend that any new commercial sanitisation processes be validated using industry-relevant conditions of sanitiser concentrations, contact times and other variables (such as organic load) on inoculated whole melons.

9 Review of prevalence, growth rates, and internalisation of *L. monocytogenes* in/on whole rockmelons from primary production to consumption

Our review (*see* Section 8) indicates that there is currently little evidence that current sanitisation interventions can reliably eliminate concentrations of >2 $\log_{10}$CFU *L. monocytogenes* from whole rockmelons. As such, risk assessment to identify potential additional risk management options should be undertaken on each farm or at least the state or national level. However, the risk assessment process requires information about the prevalence and concentration of *L. monocytogenes* on whole rockmelons and the potential for growth throughout the farm-to-fork rockmelon supply chain to be available to risk assessors. Therefore, the objective of this review was to analyse and summarise the published data on prevalence, growth rate, and internalisation of *L. monocytogenes* and rockmelons to identify what information is available but also data gaps and future research needs.

9.1 Prevalence of *L. monocytogenes* in/on whole melons from primary production to consumption

The literature review identified eleven studies that assessed the prevalence of *L. monocytogenes* in/on rockmelons or their environments pre-harvest (in the field), post-harvest (in the packhouse or transport), or retail. We did not include the results of outbreak investigations. The results of those studies are summarised below.

- Four studies provided analyses of the pre-harvest environment and melons from the field (Table 28), with no study reporting the detection of *L. monocytogenes* (a "positive") result among 560 samples in four nations.
- Four studies surveyed melons or environmental samples at different points during processing (Table 29), with three positive samples detected among > 363 samples.
- Seven studies tested whole melons at retail or wholesale level, across five countries, with one positive result among 3293 samples (Table 30).

Table 28. Studies that have assessed the prevalence of *L. monocytogenes* in the melon preharvest and harvest environment.

Reference	Country	Sampling Year	Sample type (size)	Sample location	Prevalence
Johnston et al. (2005)	USA	2000-2002	Rockmelon (25g)	Field	0/36
Park et al. (2013)	Korea	2012	Seed (2g)		0/36
Park et al. (2013)	Korea	2012	Plant leaf (5g)		0/36
Park et al. (2013)	Korea	2012	Irrigation water (25ml)		0/6
Park et al. (2013)	Korea	2012	Rockmelon (25g)	Field	0/18
Park et al. (2013)	Korea	2012	Soil (25g)		0/18
Heredia et al. (2016)	Mexico	2011 – 2012	Rockmelon (Rinse)	Field	0/106
Heredia et al. (2016)	Mexico	2011 – 2012	Worker hands (Rinse)	Harvest	0/70
Heredia et al. (2016)	Mexico	2011 – 2012	Soil (25g)		0/38
Heredia et al. (2016)	Mexico	2011 – 2012	Source water		0/16
Heredia et al. (2016)	Mexico	2011 – 2012	Irrigation water		0/38
NSW DPI (2020)	Australia	2019	Melons (Rinse)	Field	0/125
NSW DPI (2020)	Australia	2019	Water irrigation channel		0/17

Notably, the data for the Australian production environments or whole melons are from the NSW region only. Those results support the anecdotal evidence that the testing undertaken by Australian producers indicates a low prevalence of *L. monocytogenes.* Given that microbial risk assessment and subsequent risk management requires an understanding of the level and frequency of contamination on the incoming product, and potential changes during processing, transport and consumer handling, it is not possible to undertake an informative risk assessment for listeriosis from Australian rockmelons currently using Australian prevalence data from multiple production regions. Furthermore, potential changes in the prevalence of *L. monocytogenes* due to season, weather events, growing region, or other factors are unknown but could potentially significantly influence risk (Pang et al., 2017).

Table 29. Studies that have assessed the prevalence of *L. monocytogenes* in the melon post-harvest environment.

Reference	Country	Sampling Year	Sample type	Sample location	Prevalence
Johnston et al. (2005)	USA	2000-2002	Rockmelon (25g)	Immediately after wash	0/3
Johnston et al. (2005)	USA	2000-2002	Rockmelon (25g)	Immediately after rinse	0/15
Johnston et al. (2005)	USA	2000-2002	Rockmelon (25g)	Conveyor	0/18
Johnston et al. (2005)	USA	2000-2002	Rockmelon (25g)	Box	0/18
Johnston et al. (2006)	USA	2002-2003	Rockmelon (25g)	Conveyor/wash	0/36
Johnston et al. (2006)	USA	2002-2003	Rockmelon (25g)	Box	0/6
FDA (2015a)	USA	2013	Various	Various	2/Unreported [a]
NSW DPI (2020)	Australia	2019	Boot swabs	Various	1/12
NSW DPI (2020)	Australia	2019	Various environmental swabs[b]	Various	0/67
NSW DPI (2020)	Australia	2019	Melons (Rinse)	Various	0/188[c]

a – two positive samples were reported to be taken from the conveyor at a packhouse. The total number of samples taken was not reported.
b – swabs from conveyor belts, rollers, walls, packing tables, cleaning equipment, and drains.
c – 40 unwashed and 148 washed

L. monocytogenes in the environment may be widely dispersed as single cells or concentrated in places such as rotting vegetation or other niches. However, given suitable conditions (*see also* Section 3.1) *L. monocytogenes* may grow and be disseminated more widely, *e.g.*, into the packhouse and processing environment. For this reason, unless frequent or intensified environmental testing is undertaken following significant weather (rainfall) events and in places where the bacterium is more likely to be present, accumulation, and colonisation of *L. monocytogenes* in the processing environment may go undetected. This accentuates the need to more fully understand the (apparently rare) circumstances that have led to the three known outbreaks of listeriosis from rockmelons.

Table 30. Studies that have assessed the prevalence of *L. monocytogenes* on whole melons sold at retail or wholesale.

Reference	Country	Sampling Year	Sample type	Sample location	Prevalence
Suslow (2012)	USA	2012	Composite rind samples	Wholesale	0/1800
Park et al. (2013)	Korea	2012	Rockmelon (25g)	Retail	0/27
Denis et al. (2016)	Canada	2009 - 2013	Whole rockmelon (Rinse enriched)	Retail	0/140[a]
Li et al. (2017)	USA	2015	Rockmelon (Rinse)	Retail	1/16
Esteban-Cuesta et al. (2018)	Germany	2014 - 2015	Rockmelon (25g)	Retail	0/147
Zhang et al. (2018)	USA	2014	Rockmelon (250g)	Retail	0/1075
NSW DPI (2020)	Australia	2019	Whole rockmelon (Rinse)	Wholesale	0/88

The review suggests that *L. monocytogenes* is generally present at very low levels in the rockmelon production environments.

The review identified prevalence data for NSW at the pre harvest/post-harvest level and at wholesale. This information supports science-based risk assessment and should be undertaken for all major Australian growing regions.

The team recommend surveys to determine the prevalence of *Listeria* spp. or *L. monocytogenes* on whole rockmelons and in environmental samples, relevant to risk, at relevant points in Australian rockmelon supply chains and from different geographic regions. While this is being undertaken in some parts of the industry, it would also be beneficial for a database to be established where results can be collated by state and nationally.

Research to enable the industry to assess the influence of weather events on the prevalence of *Listeria* spp. on fruit in the field and the growing environment is recommended.

9.2 Growth rates of *L. monocytogenes* on the rind or flesh of melons

The literature review identified seven published studies that report growth rates (or generation times) for *L. monocytogenes* on the rind or flesh of melons. Those studies, summarised in Table 31, include not only data but also several mathematical models that quantify the influence of temperature on the growth rate of *L. monocytogenes* on melon flesh or rinds. The models presented by Fang et al. (2013) and Danyluk et al. (2014) for predicting growth rates of *L. monocytogenes* on fresh cut pieces of rockmelon agree very closely with each other. Other growth rates on melon flesh have been reported (*see* Figure 7) and do not agree as well with the results of Fang et al. (2013) and Danyluk et al. (2014), but they may have been influenced by the growth of other microbiota: e.g., slower rates were reported at higher temperatures, which is consistent with the notion of other microorganisms competing with *L. monocytogenes* (*L. monocytogenes* has an advantage at lower temperatures because it can grow at refrigeration temperatures) but other organisms can grow faster and possibly 'outcompete' *L. monocytogenes* at warmer temperatures.

Table 31. Published studies reporting growth rates of *L. monocytogenes* on the rind or flesh of melons.

Reference	Fruit	Growth substrate	Temperature (˚C)	pH	Aw	Notes
Penteado and Leitão (2004)	Melon (*Valenciano amarelo*) Watermelon	Pulp	10, 20, 30	5.87 5.50 (WM)	NR	Primary model – linear model. Growth was observed at all temperatures, generation times faster for other melons compared to watermelon (*n.b.,* watermelons are more acidic so slower growth rates are expected).
Fang et al. (2013)	Rockmelon	Flesh pieces	4 - 43	NR	NR	Primary model - 3-parameter logistic model (Fang et al., 2012). Secondary model – Cardinal parameter model (Rosso et al., 1995), Ratkowsky 4 parameter square-root model (Ratkowsky et al., 1983), and Arrhenius-type model (Huang et al., 2011). **Typographical error identified by Danyluk et al. (2014) and confirmed in *this* study, i.e., Ratkowsky *b* parameter should be $2.24*10^{-1}$ Cardinal parameters estimated agreed closely with published literature. Observed that *L. monocytogenes* grows well on rockmelon pieces at temperatures from 4 – 43˚C. Melons pre-washed in water only.
Danyluk et al. (2014)	Rockmelon Watermelon Honeydew	Flesh pieces	4 - 25	NR	NR	Primary model - Baranyi and Roberts (1994) model Secondary model – simple Ratkowsky square-root type model (Ratkowsky et al., 1982) Very high level of agreement with Fang et al. (2013) model predictions. Corresponded also to predicted values from ComBase under similar pH and water activity values but observed growth rates were lower than ComBase predictions. Melons pre-sanitised in 200ppm chlorine 30min. So reduced background microbiota.

Hong et al. (2014)	Rockmelon	Flesh pieces	10 - 30	NR	NR	Primary models -- Baranyi and Roberts (1994) model, modified Gompertz (Zwietering et al., 1990), and Huang models (Huang, 2013). Secondary model - Arrhenius-type model (Huang et al., 2011) – note that there is an error in the model as presented in the publication – the model in the publication lacks the "(b(T-Tmax))" term that was given in the cited references (Huang, 2011, Huang, 2013). Accordingly, the model predictions did not agree with other observations at all. No significant differences in the maximum growth rate and lag phase duration between the cultures of *L. monocytogenes* with or without previous cold-adaptation treatment. Fang et al. (2013) reported a higher Δ*G*' value (2560 J/mol) for *L. monocytogenes* on fresh-cut *rockmelon* than in this study, but that model was for N_{max}, rather than growth rate.
Abeysundara et al. (2017)	Rockmelon	Flesh suspension Rind suspension	10, 22	6.5	NR	Primary model – linear model, i.e., a simple linear regression of log growth rate data, similar to Penteado and Leitão (2004). Similar generation times were reported for suspension both on flesh and rind samples.
Salazar et al. (2017)	Rockmelon	Flesh pieces Rind pieces	5, 10, 25	6.19	0.987	Primary model – Baranyi and Roberts (1994) model. Maximum growth rates and population densities significantly lower in rind than flesh. (Melons were not pre-sanitised). Rind 25˚C time to 1 log = 11.7h average of 3 strains, 5˚C = 54.72h Flesh 25˚C time to 1 log = 8.6h average of 3 strains 5˚C = 51.87h
Scolforo et al. (2017)	Canary melon	Flesh pieces Rind pieces	5 – 35˚C	5.7	>0.98	Primary model – Baranyi and Roberts (1994) model. Secondary model – Ratkowsky square root model (Ratkowsky et al., 1983). No significant difference between growth rate and lag time when inoculated on the outer rind and pulp following storage between 15 and 35˚C. (Melons pre-sanitised).

Modelled results from the studies of Fang et al. (2013) and Danyluk et al. (2014), based on observed growth rates on rockmelon flesh were compared with the ComBase[14] predictive microbiology model for *L. monocytogenes*, as also shown in Figure 7. Even allowing for a slightly acidic pH (6.2), the ComBase model predicted faster growth rates than were observed by Fang et al. (2013) and Danyluk et al. (2014). The ComBase model, however, was developed from studies in nutrient rich, laboratory broths and would not include inhibitory compounds that could be present in rockmelon, *e.g.* organic acids. Equally, *L. monocytogenes* is an amino acid auxotroph and rockmelon may not adequately supply its preferences for pre-formed amino acids, whereas they are present in the rich, protein-based laboratory media that are used to develop the growth rates reported in ComBase. Nonetheless, the differences in growth rates predicted from ComBase, and

14 ComBase is a free, internet-based, database of microbial growth rate data and predictive models that can be used to make predictions about growth rates in different foods under various conditions of temperature, pH and water activity. It can be accessed at: https://www.combase.cc/index.php/en/.

those observed by Fang et al. (2013) and Danyluk et al. (2014), are relatively small (~20% – 50% difference) but are relatively greater at lower temperatures.

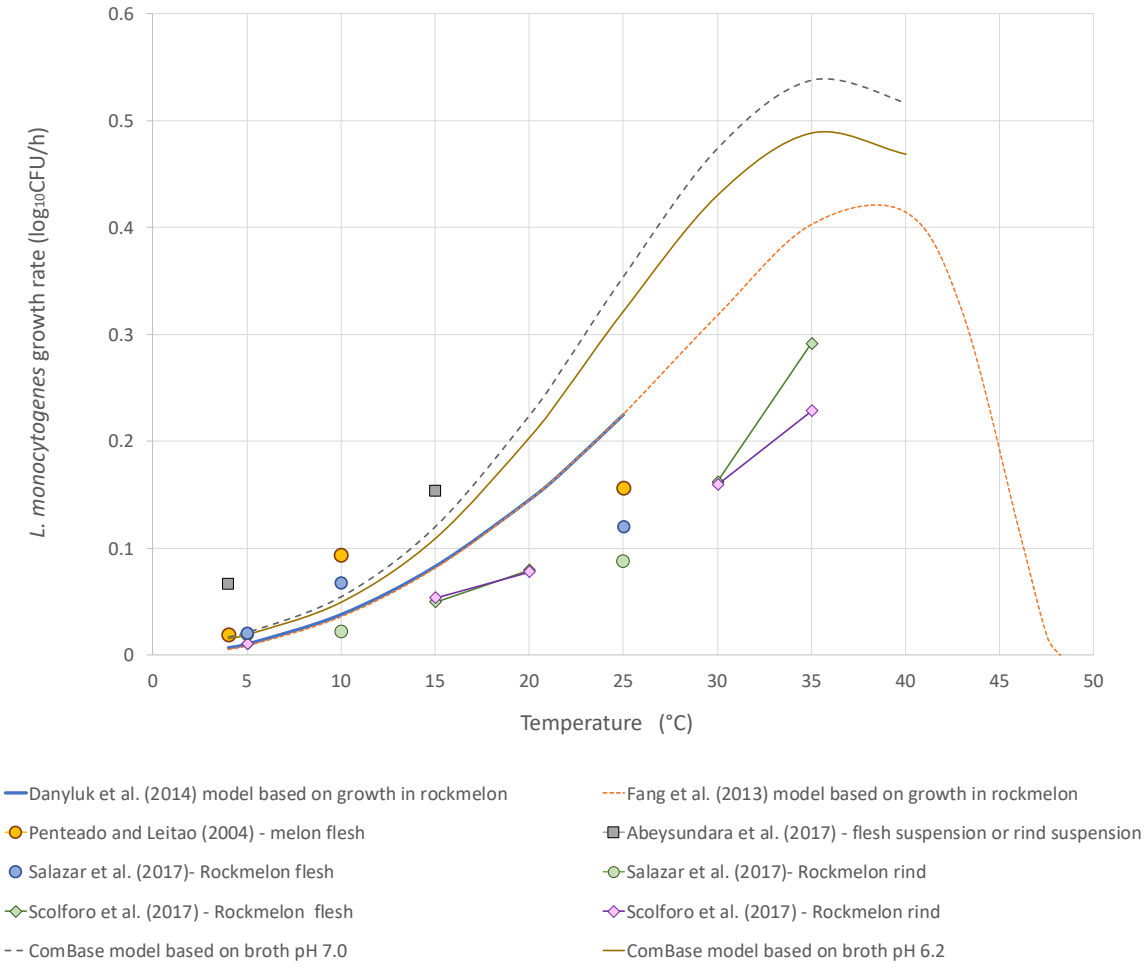

Figure 7. Observed or modelled growth rates of *L. monocytogenes* on rockmelon flesh or rind from various published studies and showing analogous predictions from the ComBase database and models of Fang et al. (2013) and Danyluk et al. (2014a).

From ComBase, and the models of Fang et al. (2013) and Danyluk et al. (2014) based on actual measurements of growth on rockmelon flesh, the expected potential growth of *L. monocytogenes* on rockmelon flesh at various temperatures over 24 h was estimated and is shown in Table 32. Those estimates show that if *L. monocytogenes* were transferred to the flesh of a cut rockmelon, *e.g.,* from the rind, and the melon was then left at room temperature (20 – 25°C), a single cell of *L. monocytogenes* could grow to levels that have a high probability of causing illness to a susceptible consumer (*see* Section 3.2) over a day. If melon flesh became contaminated with *L.*

monocytogenes (*e.g.,* transferred to the flesh of the melon from the rind, or a contaminated contact surface, or by internalisation) and was stored at 5°C it would take 2 – 3 weeks for a single cell of *L. monocytogenes* to grow to levels likely to cause illness in a *susceptible* consumer, but this is likely to be longer than the shelf life of the product. If the initial contamination were higher, the time to reach a 'high risk' dose would be shorter.

Also evident from the data and notes in Table 31 there is some inconsistency between observations of growth of *L. monocytogenes* on the rind of rockmelons in the results of Abeysundara et al. (2017), Salazar et al. (2017) and Scolforo et al. (2017) compared to the growth rates observed in rockmelon flesh by Danyluk et al. (2014) and Fang et al. (2013). In addition to the published literature, we also had access to unpublished data (M. Danyluk, pers comm., 2020) that were collected under well-controlled conditions that also enable comparison of growth on the flesh or rind of rockmelon. In those data (M. Danyluk, pers. comm, 2020), growth rates for a four-strain cocktail of *L. monocytogenes* (including outbreak strains) inoculated onto the surfaces of rockmelons were compared to growth rates obtained from the same strain cocktail inoculated on rockmelon flesh (as reported in (Danyluk et al., 2014)). In that study, no statistically significant difference in growth rates on flesh or rind was observed. Thus, from the available studies it appears that growth on the rind is possible, but there is less consensus about the rate of growth on the rind compared to the rate of growth on melon flesh. Potential reasons for these differences in observations between studies were investigated by considering, in detail, differences in the methods used by those four sets of authors, as discussed below.

Abeysundara et al. (2017) used "washed" cells of a cocktail of *L. monocytogenes* strains but inoculated them into suspensions (termed 'extracts') of either macerated rockmelon flesh or macerated rockmelon rind fractions. Importantly, by using washed cells suspensions there should have been no carry-over of nutrients from the growth medium to the experimental systems but the use of macerated rind may potentially have released nutrients from the rind into the suspension that would not be available on the intact rind surface. Similarly, these extracts were made up in large volumes of deionised water, and then sterilised by autoclaving. In doing so, the authors eliminated the probable inhibition of *L. monocytogenes* growth due to low water activity as would be expected on naturally contaminated rockmelon rind, and possibly also inhibition due to other microbiota on the rind.

In the studies of Scolforo et al. (2017), cocktails of strains of *L. monocytogenes* were grown in Brain Heart Infusion Broth (BHIB) over two cycles and then directly inoculated onto samples of rockmelon flesh or intact rind samples. However, because the inoculum was not a washed cell suspension, nutrients from the BHIB may have altered the potential for growth of *L. monocytogenes* on the rind samples and led to faster/more prolific growth than would normally occur on the outside of intact rockmelon.

In the third study we identified that compared growth of *L. monocytogenes* on rockmelon flesh and rind (i.e., Salazar et al. (2017)) the authors used canary melons (a variety of rockmelon), and spot-inoculated them with suspensions of a three-strain cocktail of *L. monocytogenes* washed in Butterfield's phosphate buffer onto intact rind surfaces or intact rockmelon flesh. In that study, there was a very large difference in the growth rates of the cocktail of strains on the rockmelon flesh, compared to the rind. Growth rates on the flesh were 2 – 3 times faster at 10 or 25°C than on the rind. Nonetheless, even in this experiment, growth *was* observed on the rind but at relatively low growth rates. The relative humidity (RH) in the storage/incubation environment in those experiments was not reported but may have influenced (potentiated) the growth of *L. monocytogenes* if it were not less than 92%, the lower limit for *L. monocytogenes* growth. Canary melons (*Cucumis melo* L. (Inodorus Group)) are a smooth skinned variety of rockmelon, and this may have also influenced the growth potential of *L. monocytogenes* on the rind compared to the fruit flesh, as observed by Salazar et al. (2017).

In the study by Danyluk (M. Danyluk, pers comm., 2020), a four-strain cocktail of strains of *L. monocytogenes* was used. The cell preparations were washed twice by centrifugation and resuspension in 0.1% peptone (which is considered not to support growth but does aid survival). A 10 µL aliquot of the combined suspension of *L. monocytogenes* was then applied to the surface (rind) of two rockmelons over an area of ~3 cm^2. The inoculated melons were then dried for one hour at ambient temperature before being transferred for storage at a range of temperatures from 4°C to 25°C. Samples were taken at appropriate intervals and *L. monocytogenes* levels determined and growth rates determined and compared to growth rates of the same cocktail of *L. monocytogenes* strains observed on rockmelon flesh (*see* Figure 8). Those studies used the Athena variety of rockmelon, which is only grown in eastern areas of the USA and is considered a 'hybrid' variety of *Cucumis melo reticulatus*. *Cucumis melo reticulatus* varieties have highly netted skin. (Conversely, *Cucumis melo cantalupensis* varieties of rockmelon, which are more typically

grown in the western regions of the USA, have a light green ribbed skin). The rockmelon involved in the 2011 USA outbreak from Jensen Farms in Colorado was, however, the 'Rocky Ford' variety, which is very highly ribbed.

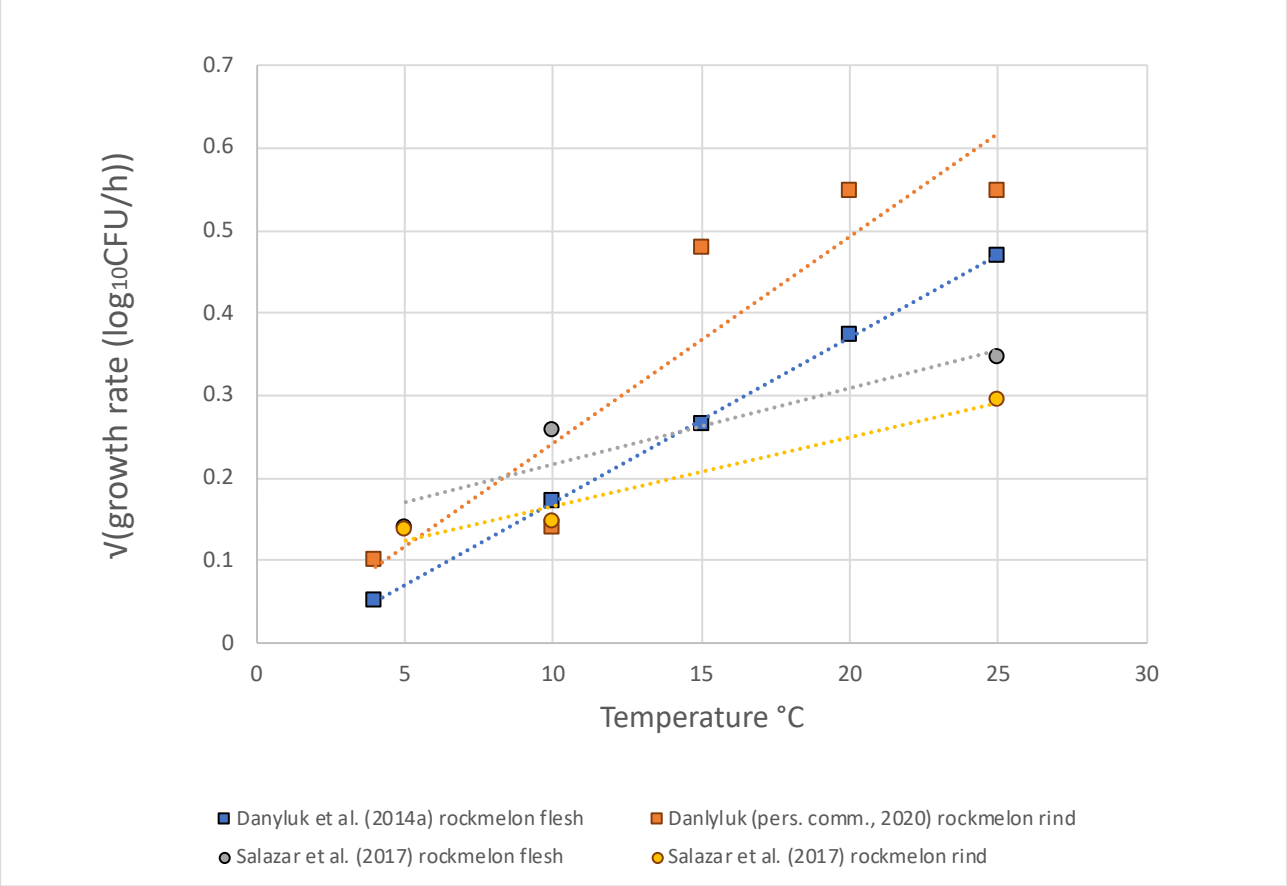

Figure 8. Observed growth rates of *L. monocytogenes* on rockmelon flesh or rind from the published studies of Salazar et al. (2017) and Danyluk et al. (2014) and unpublished data of Danyluk (M. Danyluk, pers. comm., 2020).

While the growth rates observed on the rind were more 'erratic' than those on melon flesh, there was no systematic difference in growth rates as a function of temperature. Notably, however, the growth rates observed were consistently higher than those reported by Salazar et al. (2017), as can also be inferred from Figure 7. In the Danyluk (M. Danyluk, pers. comm., 2020) study, the relative humidity in the storage environments was relatively high (90 – 97%) but was reported to be less than 0.92 at both 10°C and 25°C.

Based on the Salazar et al. (2017) observations for growth rates of three strains of *L. monocytogenes* on rockmelon flesh or rind at three temperatures, we calculated the average observed growth rates and, from that, the potential for growth of *L. monocytogenes* on rockmelon flesh or rind at three temperatures, over time (*see* Table 32). We also included the expected rates and potential growth from the Danyluk et al. (2014a) model. Over one day these differences in exponential growth rates can translate into listeriosis *risk* differences of over 1,000-fold if growth occurs only on the rind or occurs on the rockmelon flesh (*see* Section 3.3) depending on the model/data used. As discussed in Section 3.2, the ingestion of 100 cells of *L. monocytogenes* represents a low risk of causing infection, even in a susceptible consumer but ingestion of 1 million cells could, e.g., cause illness in 1% of susceptible consumers. As the dose ingested increases, however, the probability of illness increases proportionally.

Table 32. Potential growth of *L. monocytogenes* on rockmelon flesh or rind at different temperatures over 24 hrs*

Temperature (˚C)	Growth rate (log_{10}CFU /h)			Growth (log_{10}CFU) over 24 hours		
	Salazar et al. (2017)		Danyluk et al. (2014a)	Salazar et al. (2017)		Danyluk et al. (2014a)
	flesh	rind	flesh or rind	flesh	rind	flesh or rind
5	0.02	0.019	0.011	0.48	0.46	0.26
10	0.067	0.022	0.038	1.61	0.53	0.91
25	0.012	0.087	0.225	2.88	2.09	5.40

* Based on growth rates reported by Salazar et al. (2017), and Danyluk et al. (2014), and assuming no lag time for growth, and noting that the 'raw' growth rate data for growth on rind at 10°C from the Salazar et al. (2017) data appears to be anomalously slow).

Collectively the results indicate that growth on the rind is possible and at relatively high rates, particularly if rockmelons are not refrigerated or retain surface moisture or develop condensation, as was discussed in Section 6. The reported rates of growth on rind - even allowing for the known effects of temperature on microbial growth rate - seem very variable between studies. The reasons for this variation have not been elucidated in this literature review but may include the extent of the netting on the surface of the melon.

We found little information on the levels of *L. monocytogenes* on whole rockmelons at the time of consumption, but the available research demonstrates that *L. monocytogenes* can grow on the outside of rockmelons as well as on the flesh.

While the reviewed data for the growth of *L. monocytogenes* on flesh was relatively consistent and from well controlled studies, the data for growth on the rind was less consistent across studies. This is a key factor to understand in risk assessment.

We recommend research to improve quantitative knowledge of factors, such as temperature, surface moisture, relative humidity, extent of netting, or others, that influence the potential for growth of *L. monocytogenes* on rockmelon rinds throughout the supply chain, to assist in identifying the most effective risk management approaches.

9.3 Internalisation of *L. monocytogenes* into rockmelons

The potential for the internalisation of pathogens into fruits and vegetables has recently been reviewed by the FDA (2017), who concluded that pathogens can be internalised and survive in fruits. Of specific interest, internalisation of microorganisms could occur in intact fruit during dump tank washing or on the tree during heavy rain. Therefore, it is critically important for the industry to understand the potential mechanisms and probability of internalisation, specifically by *L. monocytogenes*, in *rockmelons* because current sanitisation methods will not inactivate internalised bacteria.

The review identified three studies that assessed the potential for internalisation of *L. monocytogenes* into whole rockmelons. Webb et al. (2015) assessed the ability of surface inoculated *L. monocytogenes* to internalise into rockmelons during the sanitising of rockmelons in dump tanks (20 to 22 °C). Melons were surface inoculated with *L. monocytogenes* on the rind and stem scar then immersed in water with different sanitisers, melons were then stored at 4˚C for 0 – 5 days, and the outer rind steamed before testing to ensure no cross contamination from rind to flesh. *L. monocytogenes* was isolated from the flesh of 7 of the 36 positive controls that were inoculated but not treated in water with sanitisers, and also from 17 of the 108 internal tissue samples of melons that were dump tanked in sanitiser for 8min. Non-inoculated non-treated melons were and were not positive for *L. monocytogenes*. The authors suggest that their results indicate that the similar frequency of isolation of *L. monocytogenes* in the internal flesh of both

immersed and non-immersed melons suggests that dumping is not the mode of internalisation but instead occurs from passive diffusion from the inoculum. They also observed a higher frequency of positive internal flesh samples from the stem scar compared to flesh from below the rind, and attributed this to a potential for increased porosity at the stem scar.

Macarisin et al. (2017), assessed the potential of *L. monocytogenes* to internalize in melons under experimental conditions of dump tank washing and hydrocooling. Rockmelons were prewarmed to either 42˚C or 18˚C then immersed for 30min in a three-strain *L. monocytogenes* cocktails at either 18˚C (dump washing) or 6˚C (hydrocooling). The use of a dye to visualize the uptake of water demonstrated that water was primarily entering through the stem scar and could permeate to the seed cavity in some cases. The uptake of water appeared less for 18˚C fruit compared to 42˚C. However, *L. monocytogenes* was found to internalise both clipped and full slip rockmelons with and without a temperature differential between the fruit and the wash water. However, internalisation was aided by warmer fruit entering cooler water and also if melons were full slip compared to clipped. The authors concluded that, if present in dump tank/hydrocooler water, *L. monocytogenes* can infiltrate rockmelons during dump tank washing and hydrocooling.

The study by Esteban-Cuesta et al. (2018), is the only study to report on the prevalence of *L. monocytogenes* internalised in retail rockmelons. This study assessed the internal pulp of internationally traded melons for a number of species. 147 melons (127 rockmelons and 20 Galia melons) were stored at +10˚C for up to 5 days before testing the rind and pulp of melons. No *L. monocytogenes* was isolated from either rind or pulp samples. However, 6.8% of internal pulp samples were positive for either *Salmonella* spp., *E. coli,* and *Bacillus cereus* isolates, and the authors concluded that internalisation of bacteria in melons occurred regularly.

The review identified three studies that assessed the internalisation of *L. monocytogenes* into whole rockmelons. There is some evidence for the internalisation of *L. monocytogenes* into rockmelons both with and without a temperature differential.

Due to the limited evidence available we recommend research to investigate the potential for internalisation of *L. monocytogenes* into whole rockmelons at different relevant points in the rockmelon supply chain (e.g., field, packhouse, consumer handling).

10 Environmental testing for *Listeria* spp.

End-product testing for *L. monocytogenes* in rockmelon is impractical for food safety management because, given the volume of production, even if only 1 in 1000 melons were contaminated with *L. monocytogenes*, it could still lead to an outbreak from a large processor. To reliably determine whether more than one in 1,000 rockmelons were contaminated, it would require testing ~3,000 rockmelons (Ross et al., 2011). This is clearly not feasible, but the numbers of samples required is a consequence of the statistics of testing using presence/absence methods (ICSMF, 2020).

A more practical solution, and one that has been embraced by other industries producing foods that are eaten without a final kill step (e.g., cooking) but might be subject to contamination with *L. monocytogenes*, is to set up an 'environmental monitoring' program. An environmental monitoring program aims to be able to quickly detect possible changes in the incidence of *L. monocytogenes* in the packing or food processing environment. That is because experience in other food industries has shown that detectable increases in the background levels of *L. monocytogenes* in the packinghouse or processing factory (i.e., a higher than usual prevalence) are a good predictor that there is loss of control in the normal operation of the process and that it might lead to contamination of product with *L. monocytogenes* and the potential for an outbreak.

In environmental monitoring programs for *L. monocytogenes*, the tests used usually are directed to detect any *Listeria*, termed "*Listeria species*" ("*Listeria* spp.") rather than *L. monocytogenes* specifically. The reasons for this approach are: i) testing for *Listeria* spp. will be more sensitive to changes in the potential prevalence and also faster, and simpler, than testing specifically for *L. monocytogenes*, ii) because *Listeria* spp. are all quite similar in their eco-physiology, *Listeria* spp. is a good indicator of the potential for *L. monocytogenes* increase, and iii) if a food business tests for, and detects, *L. monocytogenes* on product that will be eaten without further processing to eliminate *L. monocytogenes,* they are required in most jurisdictions to inform regulatory authorities and to cease production until the source of the *L. monocytogenes* is found and eliminated. Testing for *Listeria* spp. allows the business to manage the detection 'in-house' and identify and remove the source of the problem without the need to involve regulatory authorities. However, environmental testing for *L. monocytogenes* will only be effective if the business employs GMP and GHP (*see* Section 7) relevant to rockmelon processing and that the business has a mature HACCP plan, or 'food safety plan;' that is actively implemented.

The design of an environmental monitoring program involves decisions about which sites in the processing area to test and how frequently they should be tested. The selection of sites for monitoring requires an understanding of the ecology of *L. monocytogenes* in packhouse environments and will probably require consultation with microbiological experts. More frequent sampling of appropriate sites means that potential problems are detected sooner and minimises the chance of a large quantity of contaminated product reaching the market. Also, the test method for environmental monitoring has to be inexpensive, specific, and reliable.

A complete review of all environmental testing methods for *L. monocytogenes* or *Listeria spp.* was beyond the scope of this project. However, as there was considerable industry interest in more rapid and easy methods to screen for *Listeria* spp. "in-house", i.e., as an indicator of potential contamination by *L. monocytogenes,* we have provided a summary of some methods that are both technically easy and rapid.

Importantly, however, there is a risk associated with the culturing of *Listeria* spp. in-house and appropriate staff training in sampling and, more importantly, in disposal of potentially contaminated material (i.e. positive tests), is essential. Examples of authoritative advice regarding environmental testing include that provided by NSW DPI (NSW DPI, 2019b; NSW DPI, 2019a) and United Fresh Food Safety & Technology Council (2018) and should be consulted so that the inherent limitations are well understood.

There have been instances where large scale, on-going contamination by *L. monocytogenes,* may have been identified earlier had regular and well-planned environmental testing been undertaken. The risk/benefit consideration of environmental testing will need to be considered by each producer individually. We recommend consulting with specialists before undertaking environmental testing for *Listeria* spp. In particular, there is little point in environmental testing for *Listeria spp*. unless the business is already applying 'Good Manufacturing Practices' and has a mature HACCP/food safety program in place and that is reliably implemented. The use of more generic tests such as ATP indicators may be less "risky" for individual business, and potentially just as effective, depending on the specific aim of the testing regime.

The following section presents some commercially available environmental testing tools for *Listeria* spp.

Hygiena™ InSite *Listeria* environmental swabs

This product is an all-in-one environmental swab that includes a chromogenic medium to determine the presence of *Listeria* spp. via a colour change after incubation. The swab is taken and replaced into the tube that initially contained it. The tube is sealed, and a section of it snapped off to allow the chromogenic media to mix with the material on the swab. The tube is then incubated at 37˚C for 24 – 48h, and presumptive positive results are identified by a change from yellow to brown/black. Sterilisation before disposal of the tubes is achieved by either autoclaving, incineration, or soaking in bleach for 1h. In addition to the purchase of the swabs, there is a need to purchase an incubator, but these can be purchased from the manufacturer of the swabs as part of the overall package. This is an AOAC approved method: Certificate Number 04051.

Product information: https://www.hygiena.com/insite-listeria-food-and-beverage.html

Product use: https://www.youtube.com/watch?v=5xWgPjeaGVg

3M™ Petrifilm™ Environmental Listeria Plates

This product involves the collection of an environmental swab that is then added to a diluent, mixed, and then poured onto the 3M™ Petrifilm™ Environmental Listeria Plate which are premade agar set between films for easy use. The film plates are then incubated for 20 – 30h, and results can be quantitative. This method requires the use of a pipette, an incubator, and an autoclave for sterilisation before disposal. It involves considerable manual handling and specialised equipment, and the interpretation of the results requires more expertise. This is an AOAC performance tested method: Certificate Number 030601.

Product information: https://www.3m.com/3M/en_US/company-us/all-3m-products/~/PETRIPA-3M-Petrifilm-Environmental-Listeria-Plates/?N=5002385+3293785686&rt=rud

Product use: https://www.dailymotion.com/video/x2szebz

CONTAM SWAB Listeria

This product is an all-in-one environmental swab that includes a chromogenic medium to determine the presence of *Listeria* spp. *via* a colour change after incubation. The swab is taken

and replaced into the tube, pushing the swab into the chromogenic media. The tube is then incubated at 37˚C for 24 – 48h, and presumptive positive results are identified by a colour change in the media from yellow to black. Sterilization before disposal of the tubes can be achieved by autoclaving, or incineration. This is not an AOAC performance tested method.

Product information: http://www.liofilchem.net/en/pdf/6553010_contam_swab.pdf

Hyserve Listeria Swab

This product is an environmental swab that includes an 'enhanced' aesculin medium to determine the presence of *Listeria* spp. via a colour change after incubation. The swab is taken and then placed into the tube with the media. The tube is then incubated at 37˚C for 24 – 48h, and presumptive positive results are identified by a colour change from straw colour to black. Sterilization before disposal of the tubes can be achieved by either autoclaving or incineration. This is not an AOAC performance tested method.

Product information: https://hyserve.com/produkt.php?lang=en&gr=3&pr=32

Listeria Transwabs ®

This product is an environmental swab that includes an enhanced aesculin media to determine the presence of *Listeria* spp. via a colour change after incubation. The swab is taken and placed into the tube with the media. The tube is then incubated at 37˚C for 24 – 48h, and presumptive positive results are identified by a colour change

from straw colour to black. Sterilization before disposal of the tubes can be achieved by either autoclaving, or incineration. This is not an AOAC performance tested method.

Product information: https://www.amsl.com.au/362/listeria-salmonella-and-coliforms-transwabs

11 Limitations of the review

There are some potential limitations to the literature review process used in this scoping study to identify the publications that were reviewed and assessed by the project team to draw conclusions and make recommendations.

The literature search was conducted with defined search strings and, while great deliberation and care were taken to identify and include all relevant terms and logical tests ('Boolean operators'), it is possible that some authoritative documents may not have been identified.

We searched two large international bibliographic databases (Web of Science and Scopus) but publications not included in those databases/search engines may not have been identified. However, references from papers that were reviewed were also consulted and added to the database. While we attempted to search the "grey literature" thoroughly, some references not available online may have been missed. Furthermore, only documents written in English were assessed: relevant publications in other languages have not been identified or included.

The literature review only identified data for interventions against pathogens *other* than *L. monocytogenes* when they had been directly compared to inactivation of *L. monocytogenes* in a single study/publication. The reason for this is that each pathogen has its own characteristic susceptibility to interventions and, while in most sanitisation studies *L. monocytogenes* was shown to be more resistant than *E. coli* or *Salmonella*, spp., the relative differences are not the same across different species. As such, our main focus was on the effects of sanitisers against *L. monocytogenes* or where a direct comparison with other pathogens was reported. This means there is data for the effects of 'interventions' on other pathogens on whole rockmelon in the published literature that was not considered to be within the scope of this review.

As discussed in Section 8.3, interventions other than those mentioned in this review may be available commercially but not reported in the published literature. Such methods were not included in the review because there are no published results evaluating those interventions *specifically* for the reduction of *L. monocytogenes* on whole rockmelon surfaces. As we have inferred that *L. monocytogenes* is generally more resistant to sanitisers and that rockmelons, because of their netted exterior appear, to be harder to sanitise than other (smooth-surface) produce, any method that is to be used in the rockmelon industry must be validated for the

specific combination of *L. monocytogenes* and rockmelon cultivar for the industry to have confidence in the efficacy of the method for their specific purpose.

The review included papers that assessed a variety of interventions, but the quality and reliability of the data presented were not systematically assessed because the primary goal was a scoping study to describe the breadth of research. Therefore, the differences in study designs and parameters and the influence this had on the results of specific studies, and how this affects the interpretation across studies, was not able to be rigorously quantified as would be the case with a systematically designed and executed experiment. However, the following general comments describe the limitations of the literature assessed in this study.

Firstly, many intervention studies do not identify the melon cultivar used. This limits the ability to discern differences due to cultivar (e.g., smooth vs. netted). The majority of studies do not test the effectiveness of the sanitiser on pathogens inoculated both on the rind and at the stem scar. This is important because several studies have reported that interventions are not as effective at the stem scar and this is a limitation to the interpretation of results for the rind only.

In most inoculation studies, melons were purchased at retail and therefore do not represent melons directly harvested from the field, e.g., the melons at retail have already been through the washing processes, which may have preferentially eliminated some groups of bacteria, had opportunity for contamination by other organisms not representative of freshly harvested fruit, and have experienced conditions in which the growth of other bacteria might be favoured. This means that it is difficult to determine the effectiveness of sanitisers with different organic loads that would be expected to come from the field. Another limitation is that the majority of studies have been undertaken in a laboratory setting, and do not assess the effectiveness of sanitisers with commercially relevant application methods, such as spraying *vs.* dumping and with the combination of brushing.

Finally, the ability to determine a 5 $\log_{10}$CFU decrease can be difficult methodologically, due to the limited attachment of *L. monocytogenes* (or other pathogens) to the surface of rockmelon and the limit of detection used in the sampling methods employed. For example, if only a 5 $\log_{10}$CFU/cm^2 attachment is achieved and a method with a 1.3 $\log_{10}$CFU LOD is used, the method can, at best, only ever demonstrate a ≥ 3.7 $\log_{10}$CFU decrease, even if the sanitiser was more effective.

12 Conclusions and recommendations

The Objectives of this project are detailed in Section 2. In this Section we evaluated our literature research, and findings presented in previous Sections, against those stated objectives and developed conclusions and recommendations based on those findings.

12.1 Factors that contribute to foodborne illness outbreaks of listeriosis linked to rockmelons

Listeriosis outbreaks from rockmelon are rare, both within Australia and internationally (salmonellosis and norovirus outbreaks, with rockmelon as the source, are far more common on an international scale). There have only ever been three listeriosis outbreaks from rockmelons reported in the international literature, but all resulted in fatalities. Two of those outbreaks occurred in Australia, and one in North America.

Although listeriosis outbreaks from rockmelons are rare, investigations after the 2018 Australian outbreak suggested that high frequency contamination of rockmelons with *L. monocytogenes* contributed to the outbreak. That high frequency (but low level) contamination possibly occurred in the field following adverse weather events (heavy rainfall in December prior to harvest, followed by dust storms) (NSW DPI 2018b) or possibly arose from contamination of rockmelons in the packhouse after colonisation of the packhouse by *L. monocytogenes* following those weather events. In the 2011 USA outbreak, the multiple strains of *L. monocytogenes* in the packhouse were potentially introduced by: low level contamination in the field followed by inadequate sanitisation allowing colonisation of the packhouse; external sources including contaminated equipment brought into the packhouse, or contaminated trucks being brought close to the packhouse to take away damaged fruit for use as animal feed.

Additionally, in the 2011 USA outbreak, because the rockmelons were washed but were not adequately cooled, it was suggested that the damp, warm, fruit might have created conditions that allowed growth of *L. monocytogenes* on the melon rind (FDA, 2012a; FDA, 2011a). Section 9.2 considered the potential for *L. monocytogenes* growth on the rind of rockmelons.

The potential for colonisation of packhouses (which is well-known from businesses that produce "ready-to-eat" foods and present somewhat similar *L. monocytogenes* risk management challenges as do rockmelons) means that growers/packhouse managers need to be aware of, and

respond to, unusual weather events that could cause contamination of fruit in the field, or other unusual circumstances that could introduce contamination into the packhouse. The responses might involve not harvesting the affected fruit, increased sanitiser contact time for potentially contaminated fruit during processing, or intensified packhouse environmental (hygiene) monitoring, e.g., for microbial contamination levels.

As noted earlier, investigations after the 2011 USA listeriosis outbreak from rockmelons and the 2018 Australian outbreak, which were both linked to single packhouses in each nation, indicated that a very high proportion of fruit at retail was contaminated with the outbreak strain(s). Despite that each outbreak lasted for 2 – 3 months and that, during the outbreak period each business potentially produced and sold 10s of millions of rockmelons before the source of the outbreak was identified and stopped, cases of listeriosis from consumption of those melons appear to have been extremely rare and were, anecdotally, of the order of 1 case per million melons. In both outbreaks, the people infected all had identifiable predisposing conditions that made them susceptible to listeriosis. In the USA outbreak, it was noted that the majority of patients remembered eating rockmelons on multiple occasions during the period leading to their infection. In the USA outbreak (147 cases), 86% of cases were aged 60 years or more, with a median age of 77 years. Seven cases (~6%) were pregnancy related or neonates. In the 2018 Australian outbreak (22 cases), the average age of cases was 70 years and with one mother-foetus pair.

The risk of a susceptible person, or any other person, of developing listeriosis is influenced by how often they are exposed to *L. monocytogenes* in the food they eat, and the number of *L. monocytogenes* cells they eat in a meal. Thus, while consumer education about food choices (particularly for the YOPI group) can reduce their chances of exposure (and has already been implemented (NSW DPI, 2018b)), minimising the contamination of rockmelons with *L. monocytogenes* and ensuring that, if *L. monocytogenes* is present, its growth (i.e., its increase in numbers over time) would be minimised, are key to managing and minimising the risk of listeriosis from rockmelons.

Compounding the threat from listeriosis to the rockmelon industry are three factors. Firstly, as food businesses become larger, even if they have excellent hygiene and even if fruit is only sporadically contaminated with *L. monocytogenes*, more people will be exposed and some may become ill and seek medical attention that will be notified to national food safety authorities (e.g.

OzFoodNet). Secondly, modern epidemiological tools (e.g., rigorous national and international foodborne illness surveillance networks and, in particular, Whole Genome Sequencing technologies) mean that outbreaks from a common source can be identified across geographic regions and across time, even if only a handful of people are affected. Thirdly, the proportion of the population in developed countries that have compromised immune systems is increasing, mostly because we are living longer, but also because the number of people living with illnesses or receiving therapies that reduce their immunity is increasing through advances in medical technology. Therefore, adoption of GAP and GMP/GHP processes (*see* Section 7), implementation of informed 'food safety plans'/HACCP schemes, temperature and moisture control of rockmelons in distribution to minimise growth on, or in, rockmelons, and hygiene monitoring of packhouse environments in combination with fruit sanitisation will probably provide greater overall food safety assurance than simply implementing processes that could achieve higher levels of inactivation of *L. monocytogenes* on rockmelons, i.e., because the contamination could also occur *after* the disinfection process from a colonised site in the factory.

12.2 Technologies for inactivation of *L. monocytogenes* on the surface of whole rockmelons

In response to industry requests for information on sanitiser efficacy, while it is clear that sanitisers make an important contribution to product safety, our literature review and analysis revealed limited consistent evidence to determine the efficacy of sanitisers currently used in Australia specifically to kill or remove *L. monocytogenes* on the surface of whole rockmelons (*see* Section 8). In response to specific industry queries, there is no evidence that sanitisation treatments currently used by the Australian rockmelon industry will reliably achieve > 3 $\log_{10}$CFU reductions of *L. monocytogenes* on the surface of whole rockmelons.

From the available data we reviewed and summarised, *L. monocytogenes* appears to potentially be generally more resistant to sanitisers than *E. coli* or *Salmonella,* so that use of sanitisers will also provide useful protection against other, potentially more prevalent, food-borne pathogens.

The 'bacterial kill' achieved by a sanitiser depends on factors such as the type of sanitiser itself, pH, temperature, organic matter, the commodity, and the target organism. In routine operation, concentration and contact time with the fruit are fundamental to sanitiser efficacy. We concluded that there is insufficient research, regarding both product quality and safety, to specify

recommendations for optimal contact times, specifically to kill *L. monocytogenes* on the surface of melons, for currently used sanitisers. Nonetheless, in the absence of more evidence the results support the recommendations of NSW DPI (2019b) for chlorine (100ppm), peroxyacetic acid (80ppm), and chlorine dioxide (aqueous) (5ppm) contact times of 2 minutes. However, due to the potentially limited efficacy of those sanitisers, as demonstrated in this scoping study, rockmelon food safety management will also require the consistent implementation of a whole-chain approach.

We identified and summarised research concerning a number of potential alternative sanitisation methods in response to industry desire to identify potential sanitisers/systems that can produce > 3 log_{10}CFU reductions in *L. monocytogenes* on the surface of whole rockmelons. Several promising technologies (including, but not limited to, X-rays, octenidine dihydrochloride, hot water, superheated steam, and dry steam) have been investigated and reported to produce > 3 log_{10}CFU reductions in *L. monocytogenes* on the surface of whole melons. However, due to limited research, cost, practicality, and other considerations, not all of these will be relevant for the Australian industry. Determination of cost/benefit for these proposed treatments was beyond the scope of this review. However, we have provided general indications of the potential benefits and limitations in this report for all sanitisers/treatments.

12.3 Research recommendations

12.3.1 Whole-of-supply chain risk management strategies

The previous publications "Melon Food Safety: A Best Practice Guide for Rockmelons and Specialty Melons" (NSW DPI, 2019b) and "Melon food safety toolbox: Practical resources for implementing best practice"(NSW DPI, 2019a), prepared by Dr Sukhvinder Pal (SP) Singh from NSW Department of Primary Industries, represent the most relevant and recent comprehensive advice provided to the Australian melon industry and should be reviewed by all stakeholders.

To minimise the risk of *L. monocytogenes* from Australian rockmelons we recommend that further research should:

- further develop and communicate a holistic risk management strategy that includes growers assessing and responding to adverse weather events, or other unusual

circumstances, and more effective and reliable hygienic handling of fruit from the field and during processing and transport

- determine the prevalence of *Listeria* spp. or *L. monocytogenes* on whole rockmelons and in environmental samples, relevant to risk, at different points in Australian rockmelon supply chains and from different geographic regions. While this is being undertaken in some parts of the industry, it would be beneficial for a database to be established where results can be collated by state, and nationally, to be able to demonstrate with confidence to risk assessors and consumers the currently apparent low prevalence of *L. monocytogenes* on rockmelons and in rockmelon growing sites in Australia

- investigate the potential for internalisation of *L. monocytogenes* into whole rockmelons at different points in the rockmelon supply chain (e.g., field, packhouse, consumer handling)

- assess the potential influence of weather events on the prevalence of *Listeria* spp. on/in fruit in the field and the growing environment and the potential persistence of *Listeria* spp. both in the soil and on whole melons in the field under different weather conditions. This assessment should include collaboration with farmers/producers regarding current practices to help frame science-based risk management decisions regarding harvest after 'adverse' weather events

- further investigate the ability of *L. monocytogenes* to colonise rockmelon packhouses from environmental sources or contaminated fruit

- improve quantitative knowledge of factors, such as temperature, surface moisture, relative humidity, extent of netting, or others, that influence the potential for growth of *L. monocytogenes* on rockmelons and how those factors vary throughout the supply chain

- investigate whether regular "in-house" environmental monitoring (both factory and growing environment) is feasible and will reduce listeriosis risk from rockmelons, and if so, develop specific guidance on environmental testing programs including methods, sites, and frequencies.

12.3.2 Technologies for inactivation of *L. monocytogenes* on the surfaces of whole rockmelons

To optimise the application of sanitisers on whole rockmelons as part of a whole-of-supply chain approach to minimise the risk of listeriosis from Australian rockmelons we recommend:

- research to determine minimum contact times at relevant concentrations for currently used sanitisers specifically to inactivate *L. monocytogenes* on the surface of whole melons, with consideration of the level of risk reduction both to consumers and the industry against practicality, economic, legal, and melon quality considerations

- validate commercial sanitisation processes using industry-relevant conditions of sanitiser concentrations, contact times and other variables (such as organic load) on inoculated whole melons

- *not* pursuing research into low penetration surface treatments such as UV and other light treatments, alone. However, in hurdle applications (using combinations of methods) there may be an application for these methods and, in general, research into the application of multiple hurdle/sanitisation technologies *is* recommended

- re-evaluating and initiating further research into methods that have demonstrated relatively high effectiveness against *L. monocytogenes* such as ozone, X-ray, octenidine dihydrochloride, hot water, superheated steam, and dry steam including determination of their costs versus benefits

- determining the efficacy of high penetration technologies, such as X-rays, to eliminate potential internal contamination of melons by *L. monocytogenes*

- future intervention studies should:

    - indicate the variety of melon used

    - assess effectiveness against multiple pathogens (e.g. *L*. monocytogenes, *Salmonella,* and *E. coli*) in parallel because they have been shown to have different resistance

- assess both the rind and the stem scar following inoculation and treatment because the efficacy of sanitisers has been shown to differ at these sites
- assess the effectiveness of treatments at multiple times after inoculation to determine the effect of biofilm formation on the effectiveness
- assess melon quality in parallel with pathogen inactivation tests to ensure that treatments are commercially viable
- perform re-inoculation and growth studies on whole melons after treatment to determine the capacity of *L. monocytogenes* to re-contaminate fruit from environmental sources
- use industry-relevant contact times, determine the effect of increasing levels of organic matter on efficacy, and apply inoculation and treatments to whole melons (rather than rockmelon portions).

12.4 Final comments

The rare outbreaks of listeriosis from rockmelons seem to be associated with a change in conditions in the field or the packhouse that introduce and/or concentrate the pathogen. If contaminated melons from the field then pass through, or overwhelm, the sanitising systems and no environmental monitoring or sufficient cleaning regimes are implemented, *L. monocytogenes* could then colonise the packhouse unchecked and contaminate even 'clean' melons.

As this scoping study suggests, the efficacy of most of the current sanitising systems for whole rockmelons may be limited: even if those sanitising systems are optimised, *L. monocytogenes* may not be completely removed and may persist at low levels. Therefore, it is important for all procedures prior to sanitising to reduce the likelihood of the pathogen entering the sanitising system. Moreover, following sanitising, hygiene procedures must strive to prevent recontamination of the fruit, and to reduce the potential for growth of the pathogen, and to prevent colonisation of the processing facility by pathogens from the field or via other routes.

We have identified a range of (potentially) more effective sanitisation methods that warrant further research due to the potential they offer for improved risk reduction for both consumers and the industry. However, all will have limitations, and from our review of available literature and

expert opinion their overall effectiveness on rockmelon food safety will depend on the implementation of a vigilant and whole-of-supply-chain approach to food safety throughout the industry.

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14 Appendices

Appendix 1: Search terms for databases

The following search strings were used to search Web of Science and Scopus on 2 October 2019.

TI= Listeria AND cantaloupe
TI= (Listeria) AND (pre-harvest)
TI= Internalization AND cantaloupe
TI= internalization AND TS=(listeria OR *Salmonella* AND cantaloupe)

Topic search= (*Listeria*) AND *(farm OR harvest* OR field OR production OR pre* OR post* OR environment* OR plant* OR transport OR storage OR store OR retail OR shop* OR pack* OR factory OR process* OR practice OR hygiene OR staff OR personnel OR worker OR source OR transmission OR route OR micro* OR bacteria* OR test* OR animal* OR weather OR climate OR water* OR rain* OR dust OR soil OR compost OR amendment* OR irrigat* OR equip* OR saniti* OR disinfect* OR antimicrobial* OR antibacterial* OR phage* OR bacteriophage* OR "sodium chlor*" OR probiotic* OR "competitive exclusion" OR lactob* OR bifidobac* OR "lactic acid bacteria" OR wash* OR dump* OR rins* OR steam* OR irradiat* OR spray* OR "hot water" OR chlor* OR chill* OR cool* OR dry* OR disinfect* OR acid OR ozone OR peroxyacet* OR peracet* OR plasma OR led OR inject* OR dip* OR hydro* OR dioxide OR twinoxide OR brom* OR electro* OR fungicid* OR oxid* OR monitor* OR petrifilm OR "whole genome" OR sampl* OR detect* OR infared OR "near infared" OR novel OR internal* OR mitigat* OR intervention* OR control OR treatment* OR trial OR combination OR hurdle OR technology OR risk OR "risk assessment" OR guidance OR outbreak* OR surveillance OR "critical control point" OR inactiv* OR reduc* OR decontam* OR contamin* OR decreas* OR grow* OR survival OR predict* OR incidence OR prevalence OR concentration OR efficacy OR cost OR benefit OR quality OR shelf OR life OR sensory OR cut* OR whole OR fresh-cut OR rind OR fresh OR produce OR surface OR variet* OR cultivar)* AND *(rockmelon* OR cantaloupe* OR *melon OR melon*)*

Google Scholar: The following search string was used to search Google Scholar between 3 October 2019 and 1st January 2020:

Advanced search:

With all of the words: listeria AND cantaloupe

With at least one of the words: whole OR rind

Due to time and resource restrictions, only the first 25 pages of 20 hits each were searched to identify any studies not found in Web of Science or Scopus databases.

Appendix 2: Abstract screening, and eligibility confirmation inclusion and exclusion criteria

Abstract screening process inclusion criteria

Does the publication detail 'best practice recommendations' that have been provided to the rockmelon industry in Australia/USA/ Europe?

☐ Yes

☐ No – see next

Does this publication describe research evaluating the efficacy and/or effectiveness of interventions to control *Listeria* in melons at stages from primary production to when melons leave the "farm gate"?

☐ Yes, primary research or systematic review/ meta-analysis or risk assessment, risk profile, or other risk-based tool (include and get full article)

☐ No – see next

If "No" to the above, does this publication describe information relevant to the growth rate, prevalence, or concentration of *Listeria* in/on rockmelons from primary production to consumption?

☐ Yes (include and get full article)

☐ No – see next

If "No" to the above, is the publication relevant to discussion about aspects covered in the background or limitations of the review, or interventions from transport through to consumption that are beyond the scope of the review.

☐ Yes (save to relevant file) These will not be subject to eligibility confirmation or characterisation.

☐ No (save to file 'excluded')

Eligibility confirmation against exclusion criteria (to be further developed following screening process)

For publications describing *Listeria* or *Salmonella* outbreaks in rockmelons, exclude if any of the following is true:

- Duplicate reference
- Not available in English
- Reports an outbreak for a commodity other than melons
- Reports an outbreak associated with a pathogen that is not *Listeria* or *Salmonella*
- Is not a review article of *Listeria* outbreaks associated with melons
- Not peer reviewed or not authored by government, regulatory, or authoritative body.
- Irrelevant to research question

For publications detailing 'best practice recommendations' that have been provided to the melon industry in Australia/USA/ Europe?

- Duplicate reference
- Not available in English
- Not authored by a government, regulatory or authoritative body
- Not relevant to research question

For publication describe research evaluating the efficacy and/or effectiveness of interventions to control *Listeria* in melons at stages from primary production to when melons leave the "farm gate", exclude if any of the following is true:

- Duplicate reference
- Duplicated results from another reference
- Not available in English
- Review article
- Not relevant to research question
- Does not provide specific results for application of the intervention against *Listeria* species ***AND*** melons
- Does not provide results from the rind of melons
- Provides results out of scope, e.g., intervention applied after melons leave the farm gate

For publications describing information relevant to the growth rate, prevalence, or concentration of *Listeria* in/on rockmelons from primary production to consumption, exclude if any of the following is true:

- Duplicate reference
- Not available in English
- Review article
- Not relevant to research question
- Does not provide specific results for *Listeria* species ***AND*** melons
- Does not provide results from flesh and/or rind of melons

Only for growth rate studies:

- Does not provide measurements of growth rate or generation time at specified temperatures

Appendix 3: Factors affecting the efficacy of sanitisers

The efficacy of chemical sanitisers is dictated by many factors including the target organism, and strain of the target organism, the concentration of the sanitiser (and the rate at which the sanitiser is consumed), the contact time, the temperature, the pH and the presence of other organic matter (Banach et al., 2015; Jensen, 2010; Lambert and Johnston, 2000). The influence of other organic matter is very important (because most sanitisers are oxidants and will 'attack' any organic material, not only the target bacteria), and explains why relevant studies are done using *L. monocytogenes* inoculated onto whole rockmelons, or rockmelon rind (NSW DPI (2019b) provides detailed best practice advice regarding the application of sanitisers currently used in Australia.)

The "Chick-Watson" equation (Chick 1908; Watson 1908) describes the influence of the sanitiser concentration and contact time on microbial inactivation:

$$log(N/N_o) = \Lambda_{CW} C^m t$$

where:

$log(N/N_o)$ is the change in log cell numbers during time 't' (N_o is the initial cell concentration, while N is the number surviving after time, 't')

C is the concentration of the sanitiser,

m is the co-efficient of dilution, an empirical parameter, frequently assumed to be 1, but that takes into account factors such as pH and inactivation of the sanitiser over time,

Λ_{CW} is the Chick-Watson coefficient that encompasses the effect of temperature, and specific responses/sensitivity of the target organism.

The relevance of the Chick-Watson model is that it suggests that there is a direct proportionality between log inactivation of the target organism (*L. monocytogenes*) and time of exposure to the sanitiser and the concentration of the sanitiser. Thus, contact time, and *effective* sanitiser concentration are key factors in the efficacy of chemical sanitisation of foods.

However, in many cases the kinetics of inactivation are not 'log-linear' and various alternative models of inactivation of pathogens due to sanitisers have been proposed to account for a reduction in 'kill rate' over time (e.g., Jensen, 2010; Lambert and Johnston, 2000).

Appendix 3 References

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