NSW PLANT PRODUCTS
FOOD SAFETY SCHEME

PERIODIC REVIEW OF THE RISK
ASSESSMENT 2019

NSW GOVERNMENT | Food Authority

Contents

Glossary

ACS	acidified calcium sulfate
ANZFA	Australia New Zealand Food Authority (now FSANZ)
APVMA	Australian Pesticides and Veterinary Medicines Authority
ARC	Australian Research Council
ASC	acidified sodium chlorite
BCCDC	British Columbia Centre for Disease Control
CCP	critical control point
CDC	Centers for Disease Control and Prevention
CODEX	Codex Alimentarius Commission
DAWR	Department of Agriculture and Water Resources
EFSA	European Food Safety Authority
EHEC	enterohaemorrhagic *Escherichia coli*
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FPSC	Fresh Produce Safety Centre Ltd
FSANZ	Food Standards Australia New Zealand
FSMA	Food Safety Modernization Act
GAPs	good agricultural practices
GHPs	good hygienic practices
GMPs	good manufacturing practices
HACCP	hazard analysis and critical control point
HPP	high pressure processing
HUS	haemolytic uremic syndrome
IFIS	Imported Food Inspection Scheme
ISO	International Organization for Standardization
ISO/TS	International Organization for Standardization technical specification
LAB	lactic acid bacteria
MAP	modified atmosphere packaging
MRL	maximum residue limit
NC	not consumed
NNPAS	national nutrition and physical activity survey
NNS	national nutrition survey

NP	not publishable
NSW DPI	NSW Department of Primary Industries
PAA	proxyacetic acid
PPP	primary production and processing
QA	quality assurance
qRT-PCR	quantitative real-time polymerase chain reaction
RNA	ribonucleic acid
RTE	ready-to-eat
RT-PCR	real-time polymerase chain reaction
SDS	sodium dodecyl sulfate
SPC	standard plate count
SSOP	sanitation standard operating procedures
STEC	shiga toxin-producing *Escherichia coli*
VTEC	verocytotoxigenic *Escherichia coli*
WGS	whole genome sequencing

Units of measurement

CFU	colony-forming unit
cm	centimetre
g	gram
hr	hour
kg	kilogram
L	litre
mg	milligram
mL	millilitre
MPa	megapascal
MPN	most probable number
ng	nanogram
ppm	parts per million
s	second

Executive summary

The previous risk assessment of the plant products food safety scheme was published in April 2014 (NSW Food Authority, 2014a). The 2014 risk assessment was part of a comprehensive review of food safety schemes undertaken during the 2015 revision of the NSW Food Regulation, which is required to be revised at five-year intervals. Each five-year review is conducted on an alternate basis, as either a full risk assessment or an update.

A full risk assessment is reported here containing new or updated information identified in an environmental scan for issues related to plant products that have impacted plant product food safety since 2014. Information sources included;

- published reports on foodborne illness outbreaks and recall data in Australia attributed to plant products,
- international issues arising from human illness or perceived hazards linked with plant products,
- risk assessments of plant products,
- emerging issues in the farm to consumer continuum for plant products relevant to health risk,
- research findings related to hazards in plant product production and processing,
- baseline surveys of microbiological and chemical hazards in plant products and,
- other relevant sources if identified during the above activities.

The hazard identification and main findings of the 2014 risk assessment remain essentially the same, in relation to fresh-cut raw fruit (melons), seed sprouts, vegetables in oil and unpasteurised juice products. However, the supportive evidence on these plant products has been updated in line with the findings garnered through work conducted by the NSW Food Authority and others. Whilst the 2014 risk assessment focused on the pathogen-commodity pair of *Listeria monocytogenes* and lettuce, this risk assessment for fresh-cut vegetables has been expanded to include all fresh-cut leafy green salad vegetables. This is in light of the recent outbreaks in Australia linked to these products. Additional hazards have also been identified in a number of products which currently fall outside the scope of the Plant Product Food Safety Scheme. These products are discussed within this risk assessment (Section 8) and include whole rockmelons, berries, fermented nut cheeses, plant-based meat alternatives and microgreens.

Where possible, consumption data from the 2011-12 National Nutrition and Physical Activity Survey has been included to support exposure assessments for specific plant products.

Baseline levels of microbiological contamination in plant products have been updated or identified from domestic and international surveys of raw and ready-to-eat (RTE) fruit and vegetable products. Of note are a number of recently published large international microbiological surveys from the U.S.A (Luchansky et al., 2017; Zhang et al., 2018), Canada (Denis, Zhang, Leroux, Trudel, & Bietlot, 2016) and Italy (Losio et al., 2015). In general, these surveys revealed an overall low prevalence of pathogens in most products surveyed.

Notable reports from Australia and internationally which were identified while undertaking this risk assessment, to be considered by risk managers in the following sectors include:

- *Cut melons:* A survey by the NSW Food Authority (NSWFoodAuthority, 2017b, 2017c) on the microbiological quality and handling practices of cut melon and papaya at retail level to better inform risk management

- *Seed sprouts*: Guidance document for the sprout industry by the Food and Drug Administration (FDA, 2017a), which includes a review of current sprout seed treatment methods, some of which purport a 5-log or greater reduction in pathogens on seeds

- *Melons*: The NSW Department of Primary Industries (NSW DPI) recent report ([NSWDPI, 2018](#)), which outlines the findings of the environmental investigation conducted by the NSW DPI into the food processing and handling practices on the farm implicated as the source of the 2018 *Listeria monocytogenes* outbreak. In addition, the Scientific opinion of the European Food Safety Authority ([EFSA, 2014b](#)) reports on the risk factors for melon contamination in the context of the whole food chain and the relevance of microbiological criteria.

- *Berries*: An Australian (Victoria) survey ([DEDJTR/FSANZ, 2016](#)) which reviews current food safety management practices during growing, harvesting and processing of strawberries

- *Fermented nut cheeses*: Review by the British Columbia Centre for Disease Control ([BCCDC, 2017](#)) on food safety process and guidance for manufacture of fermented nut cheeses

- *Microgreens*: Studies demonstrating systematic contamination of microgreen plants by *E. coli* O157:H7 contaminated seeds ([Xiao et al., 2015](#)) and *Salmonella* contaminated seeds and irrigation water ([Reed, Ferreira, Bell, Brown, & Zheng, 2018](#))

Globally and domestically, there has recently been significant attention focused on the safety of fresh produce and plant products. It is likely that at the time of the next review of the Plant Products Food Safety Scheme, considerable progress will have been made in domestic and international research centred on the food safety of plant products. In turn, it is anticipated that this will lead to knowledge-led development and/or improvement of existing guidelines for industry, government-based standards and policies and, potentially novel interventions and technologies to improve food safety in this sector. Specifically, research projects within the Australian Research Council (ARC) Training Centre for Food Safety in the Fresh Produce Industry are slated to be complete by 2020 and will provide new insight into the fresh produce food safety landscape within Australia.

1. Introduction

1.1 NSW Food Regulation 2015

Table 1: Licensed plant products processing businesses in NSW*

Primary approved activity	Secondary approved activity				
	Process seed sprout	Fresh cut fruit and/or vegetables	Extract and/or unpasteurised juice	Process vegetables in oil	TOTAL
Process seed sprout	8	-	-	-	8
Fresh cut fruit and/or vegetables	2	44	-	1	47
Extract and/or unpasteurised juice	-	6	12	-	18
Process vegetables in oil	-	-	-	8	8

* Data extracted from NSW Food Authority Byte licensing database on the 4th of December 2018

1.2 Australia New Zealand Food Standards Code requirements

With the exception of seed sprouts (Standard 4.2.6 Production and Processing Standard for Seed Sprouts), the Australian fresh produce sector is not regulated by means of primary production and processing standards under Chapter 4 of the Australia New Zealand Food Standards Code (the Code). Standards 3.2.2 and 3.2.3 of the Code apply to minimal processing and packing operations where these activities do not constitute primary food production. Standard 3.2.2 of the Code requires that a food business must take all practical measures to ensure it only accepts food that is protected from the likelihood of contamination and, must be able to identify food on its premises and where it has come from. Aspects of Chapter 1 and 2 of the Code also apply to the production and processing of fresh produce, including maximum residue limits for chemical residues and contaminants, labelling of packaged produce and the use of additives and processing aids. Non-regulatory industry-based food safety programs have been developed for the horticulture sector in Australia and implemented widely. The majority of fresh horticulture production occurs under voluntary quality assurance programs, through audited industry programs or other systems that address food safety. The effectiveness of these food safety programs relies on a commensurate level of participation, consistent interpretation of programs and treatment of audits as more than an administrative formality. The majority of Australian fresh and processed produce is grown, packed and processed under one or more internationally recognised food safety programs, such as SQF, BRC, Freshcare and GLOBALG.A.P (FPSC, 2017). Australian retailers require their international suppliers to be certified to the same level as Australian growers. However, smaller operations that do not supply to major retailers, may not be under a similar level of scrutiny and may not even have implemented a Hazard Analysis and Critical Control Point (HACCP) plan. There is provision in the Food Standards Code to implement regulation that applies to all primary producers so that they will be legally required to implement a food safety program. In 2013, Food Standards Australia New Zealand (FSANZ) proposed a broad-based primary production and processing standard for horticulture (P1015), with the aim of strengthening food safety and traceability throughout the horticulture supply chain. However, following consultation, FSANZ chose not to progress the standard in favour of non-regulatory measures (FSANZ, 2014).

1.3 Updating the 2014 Risk Assessment

This updated Risk Assessment was produced following an environmental scan for issues related to plant products that have impacted plant product food safety since 2014. Information sources included published reports on the following:

- foodborne illness reports and recall data in Australia attributed to plant products

- international issues arising from human illness or perceived hazards linked with plant products

- risk assessments of plant products

- emerging issues in the farm to consumer continuum for plant products relevant to health risk

- research findings related to hazards in plant product production and processing

- baseline surveys of microbiological and chemical hazards in plant products

- other relevant sources if identified during the above activities

In Australia there have been a number of foodborne illness outbreaks linked to plant products, which fall within (Appendix 1) and outside (Appendix 2) the current scope of the Plant Product Food Safety Scheme. In addition, a number of recalls in Australia have involved imported frozen plant products contaminated with either bacterial or viral pathogens (Appendix 3). At the Australia and New Zealand Ministerial Forum on Food Regulation (the Forum) in June 2018, Forum Ministers noted the recent increase of foodborne illness outbreaks in Australia and agreed that there is a need to reassess the food safety risk management of five high risk horticulture sectors: ready to eat, minimally processed fruits and vegetables, fresh leafy green vegetables, melons, berries and sprouts (theForum, 2018). Forum Ministers requested that FSANZ identify appropriate regulatory and non-regulatory measures for Australia to manage food safety risks in these sectors (theForum, 2018). The current Risk Assessment includes discussion of high risk plant products identified from the environmental scan conducted as detailed above, as well as all plant products belonging to the horticulture sectors noted for reassessment by the Forum. Those products which fall outside the scope of the Plant Product Food Safety Scheme, are discussed in a section titled "Other Products" (section 8).

1.4 International risk assessment and food safety research

Ranking of health risks related to food safety is generally recognised as the basis for risk-based priority setting (Van der Fels-Klerx et al., 2018). In the previous Risk Assessment (NSWFoodAuthority, 2014a), a number of published reports were cited which provided risk rankings for fresh produce commodity and pathogen combinations using data either solely from the U.S.A (Anderson, Jaykus, Beaulieu, & Dennis, 2011) or the EU (EFSA, 2013). In data derived from the U.S.A, Anderson et al. (2011) ranked leafy greens and *E. coli* O157:H7 (EHEC) first in all model iterations (Anderson et al., 2011). In a majority of iterations, *S. enterica* in tomatoes and *S. enterica* in leafy greens consistently ranked second and third, respectively (Anderson et al., 2011). In data derived from the EU, the European Food Safety Authority (EFSA) concluded that the top ranking food/pathogen combination was *Salmonella* spp. and leafy greens eaten raw followed by (in equal rank) *Salmonella* spp. and bulb and stem vegetables, *Salmonella* spp. and tomatoes, *Salmonella* spp. and melons, and pathogenic *E. coli* and fresh pods, legumes or grain (EFSA, 2013). Since this time, there have been no other published risk rankings for fresh produce commodity and pathogen combinations, apart from the findings of the EU study (EFSA, 2013) being published in another journal more recently (Da Silva Felicio et al., 2015). While these studies provide an indication of the pathogen/commodity pairs of most concern in the U.S.A and Europe, the knowledge is not necessarily directly transferrable to the Australian situation. In addition, as highlighted in the previous Risk Assessment (NSWFoodAuthority, 2014a), Australia has not experienced the same magnitude of outbreaks as the U.S.A and the EU.

Globally, considerable attention has been focused on the safety of fresh produce and plant products. The intention of the following section is not to give an exhaustive overview, but rather to highlight where there has been a concentrated effort in undertaking food safety research that may translate into applied outcomes for the Australian plant products industry. The Fresh Produce Safety Centre Ltd (FPSC) at the University of Sydney, is an industry-led, not-for-profit company established to enhance fresh produce safety across Australia and New Zealand through research, outreach and education. The first two completed R&D projects of the FPSC are the Guidelines for Fresh Produce Food Safety (FPSC, 2015a) and Understanding the Gaps: A Food Safety Literature Review (FPSC, 2015b). The Guidelines for Fresh Produce Food Safety (2015) are designed to assist growers, packers, transporters, wholesalers, retailers and others involved in the fresh produce supply chain to identify and assess potential food safety hazards (FPSC, 2015a). The Guidelines are designed to achieve greater consistency in the development, implementation and auditing of fresh produce food safety programs (FPSC, 2015a). Understanding the Gaps: A Food Safety Literature Review (2015), identified key priority research areas including gaining a greater understanding of:

- the level and type of fresh produce microbiological contamination in Australia and New Zealand

- the potential for pathogen transfer from agricultural water to produce surfaces and improved information on water quality risk assessment, testing and water source management

- the types of manures used in Australia and New Zealand composts and the pathogen types that are able to persist in these treatments

- the prevalence and persistence of *L. monocytogenes* in incidental condensate within fresh produce storage environments

- sanitiser and fungicide use patterns in Australia and New Zealand produce industries to prioritise which produce types or sanitiser and fungicide use patterns need to be evaluated for compatibility.

The FPSC is a partner in the ARC Training Centre for Food Safety in the Fresh Produce Industry, which received funding in 2016 from the ARC over four years to support food safety research. Nine research projects, which arose from the findings of the Understanding the Gaps: A Food Safety Literature Review (FPSC, 2015b), are underway across three themes; (1) on-farm environment; (2) postharvest environment and, (3) risk assessment. The FPSC is also working closely with The Center for Produce Safety in the U.S.A.

In consideration of the large number of food poisoning outbreaks in the U.S.A attributed to fresh produce, the Food and Drug Administration (FDA) has issued regulations; two sections of which are "Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption" (FDA, 2015b) and "Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food" (FDA, 2015a). The first section, referred to as the "Produce Rule," applies primarily to raw agricultural commodities that are not further processed and that may be eaten raw. Key inputs into the produce growth environment with specific requirements include: agricultural water (including microbiological criteria for waters and a requirement to test untreated waters used for certain purposes); soil amendments (manures and compost, with microbiological criteria set for pathogens in composts); sprouts (with microbiological criteria set for pathogens in seeds, spent irrigation water or sprouts); animals in growing area (restricting access of wild or domestic animals to covered growing areas); worker training and health (preventing worker contamination of produce); and equipment, tools and buildings (establishing hygienic standards). The second section, referred to as the "Preventive Controls Rule," applies to produce that is cut, peeled, or otherwise processed. Preventive controls apply to facilities but not to farms or some kinds of on-site packing operations. The preventive controls regulations must be implemented so that identified hazards are prevented or significantly minimised, and the preventive controls must be validated, based on scientific and technical information, to demonstrate that they prevent or significantly minimise the identified hazard. In light of these new regulations, there has been a considerable research focus on addressing knowledge gaps pertaining to the implementation of these new

regulations within the produce industry. For example, a recent publication assessed the correlation between *E. coli* levels and the presence of foodborne pathogens in surface irrigation water, to give insight into how a water sampling program should be established (Truchado, Hernandez, Gil, Ivanek, & Allende, 2018). In another recent publication, citing the fact that procedures have not yet been articulated, practical guidelines were reported on validating antimicrobial washing as a preventive control for the safe production of fresh-cut leafy vegetables (Gombas et al., 2017). It is likely that there will be a continued research focus on addressing these gaps, while the produce industry within the U.S.A grapples with implementation of the new regulations.

Another area receiving considerable attention internationally, is the application of whole genome sequencing (WGS) and other genomics-based tools to drive further improvements in food safety. WGS can detect foodborne disease outbreaks and trace pathogen sources along the global food chain with unprecedented resolution. For example, WGS was recently used to define a multi-country outbreak that began in 2015 and resulted in 47 cases; including nine deaths, across Austria, Denmark, Finland, Sweden, and the United Kingdom (EFSA, 2018). WGS identified frozen vegetables that had been produced by the same Hungarian company since 2016, as the source of the outbreak of *L. monocytogenes*. The information obtained from WGS led to the finding that *L. monocytogenes* had persisted in the processing plant, despite the cleaning and disinfection procedures that were carried out. Consequently, in 2018 EFSA was requested to provide recommendations to the European Commission on the sampling strategies and established microbiological methods most appropriate for maximising the sensitivity of detection of *L. monocytogenes* in processing water and the environment of premises producing frozen fruits, vegetables or herbs as well as on the final food produced; and on the identification of critical sampling sites for environmental monitoring of *L. monocytogenes* (EFSA, Allende, et al., 2018). Seven steps were defined for a fit-for-purpose sampling strategy for the production of frozen fruits, vegetables or herbs, which will support authorities and food business operators (EFSA, Allende, et al., 2018). While WGS is currently mainly used to support outbreak investigations (EFSA, García Fierro, et al., 2018), it has the potential to offer immense benefit to the fresh produce industry as a whole. In examining the food industry's current and future role in preventing microbial foodborne illness within the U.S.A, Doyle et al. (2015) advised that food producers and processors should expand their efforts in environmental and finished product testing to aid in identifying system weaknesses (Doyle et al., 2015). Doyle et al. (2015) recommend that collection of samples for WGS would not only assist identification and traceback during outbreaks, but would also aid food processors in understanding the ecology of pathogens and to control their persistence in the environment. The transformative potential as well as existing bottlenecks in applying WGS in food safety management are well documented (FAO, 2016).

2. Risk assessment of fresh cut, raw fruit

In the plant products food safety scheme:

fresh cut fruit means any fruit that has been processed in some way (for example, by trimming, cutting, slicing, peeling or pulling apart), but is still raw.

2.1 Hazard identification – cut melons as a potentially hazardous food

The main types of melons produced (and consumed) in Australia are watermelon, rockmelon and honeydew melon (NSWFoodAuthority, 2017b). Melons are either sold to the market as whole fruits, or they are processed first. When they are processed, they are, for example, pre-cut or prepared as an ingredient for fruit salad, meaning that they enter the food trade as a RTE product. The Authority licenses businesses that manufacture fresh cut fruit and requires manufactures to have a food safety program in place. The NSW Food Safety Schemes Manual (NSWFoodAuthority, 2016b) also requires testing of every 10 batches of product to ensure that *Salmonella* and *Listeria* are absent in 25g. As outlined in the previous Risk Assessment (NSWFoodAuthority, 2014a), these measures combined appear to adequately reduce the risk.

While the scope of the NSW Food Authority's Plant Products Food Safety Scheme does not extend to food handled at retail, cutting up and packaging of melons can also be done directly by the retailer before the goods are displayed for sale. Supermarkets and greengrocers often sell melons that have been cut and wrapped in cling film on-site (NSWFoodAuthority, 2017b). Major supermarkets often refrigerate cut rockmelon, honeydew and papaya but not necessarily cut watermelon (NSWFoodAuthority, 2017b). Most greengrocers do not display these products under temperature control (NSWFoodAuthority, 2017b).

Melons, particularly netted melons (i.e. rockmelons), are recurrent vehicles of pathogens causing outbreaks of foodborne illness. Foodborne pathogens can attach to the melon surfaces and survive postharvest sanitation processing if the fruit is not washed and brushed properly in the presence of optimum concentrations of chemical sanitisers. Microbes surviving on the surfaces of the melon can then easily be transferred to the inner part of the tissue during cutting. Because rockmelons, honeydew and watermelons are low acid (high pH) fruits, they offer little or no inhibition of bacterial growth. The nutrient-laden juice of the inner tissue may also allow the proliferation of pathogenic bacteria when proper sanitation and strict temperature control are not maintained. A number of studies; reviewed in the previous Risk Assessment (NSWFoodAuthority, 2014a) and since then (Feng et al., 2017; Huang, Luo, & Nou, 2015; Nyarko et al., 2016), have shown that human pathogens may proliferate rapidly under temperature abuse conditions on fresh-cut melon. Unrefrigerated storage of cut melon is likely to be an important risk factor at retail and catering including in domestic and commercial environments (EFSA, 2014a).

The relatively low efficacy of postharvest antimicrobial interventions for rockmelons (for more detail see section 8.1) determines the critical importance of effective cold chain management of fresh-cut rockmelons (Huang et al., 2015). Huang et al. (2015) investigated the growth of *Salmonella enterica* and *Listeria monocytogenes* on fresh-cut rockmelon under different temperature abuse scenarios. To mimic an acute temperature abuse situation, inoculated fresh-cut rockmelon was exposed to room temperature (25 ± 2°C) for 4 hours (Huang et al., 2015). For *Salmonella*, exposure of inoculated rockmelon cubes to room temperature abuse for 4 h post-inoculation, resulted in a 0.39 log unit increase in cell count in comparison to the control (no temperature abuse) (Huang et al., 2015). For *L. monocytogenes*, the effect of exposure to acute temperature abuse displayed no significant log unit increase in cell count in comparison to the control (no temperature abuse) (Huang et al., 2015). Most acute temperature abuse episodes are likely terminal, where products are either consumed or discarded after exposure to abusive temperatures. However, in some cases food subjected to acute temperature abuse could be returned to cold storage for sale or consumption at a later time. Therefore, Huang et al. (2015) also evaluated the growth potential of *Salmonella* and *L. monocytogenes* on fresh-cut rockmelon during cold storage for up to 7 days, after being exposed to acute temperature abuse conditions. Delaying

refrigeration (4 h exposure to 25 ± 2°C before cold storage) after inoculation resulted in an increase in *Salmonella* population by 0.6 log unit, which was steadily maintained during the ensuing 7 day storage period. For *L. monocytogenes*, 4-h exposure to room temperature did not immediately result in a significant increase of cell counts on fresh-cut rockmelon. However, the cell counts for *L. monocytogenes* on the samples subjected to delayed refrigeration, were consistently ~0.5 log unit higher than the control when enumerated throughout the week-long cold storage. These observations strongly support time and temperature controlled storage (the 4-hour/2-hour rule) of fresh-cut melon and also highlights the potential issues associated with returning these products to refrigerators for later consumption in the home and retail setting.

2.2 Exposure assessment – consumption of melons

During recent years, the production of minimally processed fruits and vegetables has increased worldwide (Hassenberg, Geyer, Mauerer, Praeger, & Herppich, 2017). In addition, growth rates of consumption of fresh-cut produce have been estimated as 10-20% per year (Hassenberg et al., 2017). While there is no retail data available to determine the growth of the pre-cut melon product line in Australia, consumption data is available for whole melons. Consumption data for melons from the 1995 National Nutrition Survey (1995 NNS) and the 2011–12 National Nutrition and Physical Activity Survey (2011-2012 NNPAS) is shown in Table 2. Data for melons from both surveys include honeydew melon, rockmelon, watermelon (including juice) and melon not further defined. Data from the 1995 NNS does not include melon in mixed dishes as there were no mixed foods/dishes that included melon as an ingredient. Data from the 2011-12 NNPAS does include melon used in mixed dishes (e.g. fruit salad and fruit smoothies). Mean consumption results are provided for the number of consumers (people who have eaten the surveyed food product), as well as the proportion of consumers out of the total respondents (people who participated in the survey and may or may not have eaten the surveyed food product) in the population group (see Appendix 4 for respondent numbers in each population group assessed). However, care should be taken when comparing data between surveys and description of any trends across the years given the different ways in which the survey data were collected, coded and aggregated (FSANZ, 2018b). There were differences in the specificity of the recipe datasets for mixed foods/dishes for each of the surveys, with the 2011-12 NNPAS possessing a greater level of detail in foods and recipes (FSANZ, 2018b). However, in absolute terms, the total mean respondent consumption (g/day) of melons was higher in the 2011-2012 NNPAS. While the total mean consumer consumption (g/day) of melons was higher in the 1995 NNS. The total mean respondent consumption (g/day) is calculated by multiplying the total number of consumers by the consumer mean consumption (g/day) and dividing this figure by the total number of respondents.

Table 2: Proportion of different age/gender groups consuming melons and amounts consumed in the 1995 NNS and 2011-2012 NNPAS*

Sex	Age group (years)	Number of consumers		% consumers		Consumer mean consumption (g/day)		Respondent mean consumption (g/day)	
		1995 NNS	2011-2012 NNPAS	1995 NNS	2011-2012 NNPAS	1995 NNS	2011-2012 NNPAS	1995 NNS	2011-2012 NNPAS
Females	2+	368	460	5.1	7.5	233	146	12	11
	2-5	19	40	4.6	12.8	187	67	9	9
	6-12	25	53	4.0	9.5	157	186	6	18
	13-18	15	36	3.4	7.9	391	194	13	15
	19+	309	332	5.4	6.9	234	144	13	10
Males	2+	227	306	3.4	5.1	264	157	9	8
	2-5	12	33	3.2	10.2	351	134	11	14
	6-12	24	45	3.6	7.6	321	152	12	11
	13-18	11	11	2.2	2.4	224	133	5	3
	19+	180	217	3.5	4.7	253	163	9	8
Persons	2+	595	766	4.3	6.3	245	150	11	9
	2-5	31	73	3.9	11.5	251	98	10	11
	6-12	49	98	3.8	8.5	237	170	9	14
	13-18	26	47	2.8	5.1	321	180	9	9
	19+	489	549	4.5	5.8	241	151	11	9

*Based on day 1 of the nutrition survey only

2.3 Hazard characterisation – melons have been linked to foodborne illness

2.3.1   Prevalence of *Salmonella* and *L. monocytogenes* on fresh-cut melon

When melons are cut, bacteria can be transferred from the skin to the flesh. Additionally, the flesh can be contaminated with pathogenic microorganisms through hands or unclean kitchen implements (knives or chopping boards). The NSW Food Authority recently undertook a survey to investigate the microbiological quality and handling practices of cut melon and papaya at retail (NSWFoodAuthority, 2017a, 2017c). The purpose of this survey was to gather data on the prevalence of pathogenic bacteria on cut melons and papayas and the handling of these products at retail level to better inform risk management. A total of 191 samples of pre-cut melons and papayas were purchased from 45 greengrocers and supermarkets across Sydney. Samples were photographed and transported under temperature control to the laboratory and the top 1-1.5 cm layer of the cut melon or papaya was tested for Standard Plate Count (SPC), *E. coli*, *Salmonella* and *L. monocytogenes*. The microbiological quality of samples tested was very good. *Salmonella* was not detected in any sample. *E. coli* was detected in one sample of watermelon at 1,100 CFU/g and *L. monocytogenes* was detected in one sample of honeydew with a level under the limit of quantification (<10 CFU/g). Of more interest were the differing levels of SPC. SPC can provide a general indication of the microbiological quality of a food. However, it does not differentiate between the natural microflora of a food and spoilage microorganisms. It should not be used to predict the safety of the product and is influenced by the storage conditions of the product. As cut melons and papaya are a raw food it is expected that they will have a low to medium SPC. A high SPC may indicate that the product has been prepared unhygienically, stored inappropriately or is nearing the end of its shelf life. The Food Authority's Microbiological quality guide for RTE foods categorises fresh cut fruit as Category C and thus no limit has been set for an unsatisfactory SPC. In this study, only three (1.6%) samples had a SPC <10 CFU/g. These were two watermelon and a pawpaw samples, purchased during summer and stored at ambient temperature inside the store. The majority (63%) of samples had an SPC between 1,000 and 100,000 CFU/g. Thirteen (7%) samples had an SPC greater than the maximum level of quantification (30,000,000 CFU/g). These samples were all purchased in summer and consisted of eight honeydews, one papaya, three rockmelon and one watermelon sourced from eleven stores. The microbiological quality of the cut fruit tested in this survey was very good, leading to the conclusion that poor practices outside of the retail environment also contribute to outbreaks.

Surveys conducted in other countries also support the low level of bacterial contamination observed in the NSW Survey (NSWFoodAuthority, 2017b). The Canadian Food Inspection Agency conducted a retail survey between 2009 and 2013, to obtain information on the occurrence of bacterial pathogens in a wide range of produce available in the Canadian marketplace (Denis et al., 2016). Amongst the commodities tested were fresh-cut (25.2%; 815/3230) and whole rockmelons (74.8%; 2415/3230). Five of the 3230 samples tested positive for the presence of either *L. monocytogenes*, *E. coli* or *Salmonella*. The five positive samples were all collected in 2015 and included three fresh-cut rockmelon samples and two whole rockmelons. Two of the fresh-cut rockmelon samples tested positive for *L. monocytogenes* at 10 CFU/g and 160 CFU/g. One fresh-cut rockmelon sample tested positive for generic *E. coli* at 240 MPN/g, while an imported and domestically grown whole rockmelon both tested positive (detected in 25g) for *Salmonella.*

2.3.2   Melons have been linked to foodborne illness

Globally, whole and pre-cut melons are frequently implicated in produce-associated outbreaks. Since the last Plant Products Risk Assessment (NSWFoodAuthority, 2014a), there have been two outbreaks in Australia involving whole rockmelons (Appendix 2). *Salmonella* was linked to an outbreak of over 150 cases in 2016. More recently *L. monocytogenes* was linked to an outbreak of 22 cases, which included 7 deaths and 1 miscarriage, in 2018. At the time of writing, a *Salmonella* outbreak occurred in the U.S.A from April to July 2018, involving pre-cut watermelon, honeydew melon, cantaloupe, and fresh-cut fruit medley products supplied by Caito Foods (Indiana, U.S.A). This multistate outbreak led to 77 cases and 36 hospitalisations across 9 states (CDC, 2018a).

In the last Risk Assessment (NSWFoodAuthority, 2014a), a review of foodborne outbreaks associated with melons led to the conclusion that most were traceable to contamination that occurred at farm and any role played by melon cutting or unrefrigerated storage is unclear. Walsh et al. (2014) reviewed outbreaks reported in the U.S.A to the Centers for Disease Control and Prevention's Foodborne Disease Outbreak Surveillance System during 1973–2011 in which the implicated food was a single melon type, as well as published literature and records obtained from investigating agencies (Walsh, Bennett, Mahovic, & Gould, 2014). During 1973–2011, 34 outbreaks caused by a single melon type were reported, resulting in 3602 illnesses, 322 hospitalisations, 46 deaths, and 3 foetal losses. Rockmelons accounted for 19 outbreaks (56%), followed by watermelons (13, 38%) and honeydew (2, 6%). Among 20 outbreaks (59%) with available contamination information, melons were contaminated during production in 13 (65%) and at the point of service in 7 (35%). The location of preparation was reported for 29 outbreaks (85%). Among the 17 (59%) with a single preparation location, restaurant or deli (6 outbreaks, 35%), grocery store (4, 24%), and private home (3, 18%) were most commonly reported. Walsh et al. (2014) defined pre-cut melons as fresh whole melons that were sliced or cut, with or without washing, before use by the consumer or retail establishment. According to this definition, among 22 outbreaks with information available, pre-cutting of melons was reported as contributing to contamination for 17 (77%). *Salmonella* was the most common aetiology reported (19, 56%). While Walsh et al. (2014) show that the initial source of contamination in most outbreaks was during production, they emphasise that efforts to intervene at the point of service should continue; including thoroughly washing melons prior to cutting and storage at temperatures of 4°C or below.

2.4 Risk characterisation – control measures for cut melons

Melons can be subject to contamination on farm, in the packing shed, during washing (if it occurs), during distribution and during cutting. The scope of the Plant Products Food Safety Scheme does not include whole raw fruits, which are not intended to be further processed. Within this Risk Assessment, existing and future considerations for control options to reduce the burden of illness associated with whole melons (specifically rockmelons) are discussed in the section on horticultural products outside of the scope of the Safety Scheme (see section 8.1).

The results of the microbiological surveys (Denis et al., 2016; NSWFoodAuthority, 2017b) have shown that contamination of pre-cut melons with bacterial pathogens such as *Salmonella* and *L. monocytogenes* occurs only very sporadically. However, pathogen-positive pre-cut melons can cause illness in consumers and therefore represent an unacceptable food safety risk. The process of cutting melons can be considered a key risk factor because of the potential to spread pathogenic bacteria from the surface/skin of the melon to the flesh of the melon where it may be able to multiply. Control measures have been recommended by various Authorities and authors (BfR, 2013; EFSA, 2014b; NSWFoodAuthority, 2014a; Parnell, Harris, & Suslow, 2005) and include:

- use only good quality fruit, free from damage or defects that may have allowed bacteria to be internalised. Avoid fruit that has visible sunken areas or areas of mould or decay

- melons should be washed with potable water before cutting or peeling

before cutting or other processing, a further reduction in microbial contamination may be achieved by scrubbing in the presence of sanitiser or application of an alternate surface decontamination process such as hot water, steam or other treatments

- the exterior surface of rockmelon is more difficult to clean than the exterior of smooth, waxy melons such as honeydew and watermelon. Mechanical cleaning with brushing in combination with an approved antimicrobial agent is essential before the rind is cut/removed and the melon is cut or peeled

- cutting or peeling knife blades should be cleaned and disinfected on a regular basis according to written procedures to reduce the potential for cross-contaminating melons during the cutting or peeling process

- knife blade disinfecting solutions should be monitored to ensure the disinfectant is present at sufficient levels to achieve its intended purpose and does not promote the potential for cross-contamination

- pre-cut melons should be wrapped/packaged and refrigerated as soon as possible and distributed under refrigeration temperatures (i.e. 4°C or less)

- the practice of re-trimming melons should be discouraged, especially if the fruit is displayed at room temperature

- the sector should be encouraged to cut small amounts and often, especially if displaying at room temperature

- for consumers, pre-cut melon should be transported from retail/market to home as soon as possible, be kept refrigerated once home and consumed as soon as possible after removal from the refrigerator.

The NSW Food Authority's recent survey investigated the microbiological quality and handling practices of cut melon and papaya at retail level to better inform risk management (NSW Food Authority, 2017a, 2017c). As discussed above, the microbiological results revealed that the cut melons sampled in the survey were safe to be displayed at room temperature for a period of time. However, it was concluded that improvements could be made in the handling, cleaning and sanitation practices used in cutting fruits at the retail level. It was also recommended that fruits should be regularly cut throughout the day using safe methods and sold on the day they are cut. The 4-hour/2-hour rule provides retail food businesses with an alternative method of complying with Food Standards Code requirements, to keep potentially hazardous foods under temperature control while on display and during short-term storage and preparation (NSWFoodAuthority, 2015b). As a result of the Survey, the NSW Food Authority recognised a need for businesses to:

- understand the difference between detergent and sanitiser, why it is important to use both and why it's important to clean and sanitise often and between cutting different types of fruit to avoid cross contamination

- avoid the practice of re-trimming melons, especially if the fruit is displayed at room temperature

- aim to cut small amounts of fruit and often, especially if displaying at room temperature.

In line with the previous Risk Assessment (NSWFoodAuthority, 2014a), there does not appear to be significant evidence in Australia that the current practice of displaying cut melons at room temperature on the day of preparation is a significant health risk. The results of the NSW Food Authority's cut melon survey (NSWFoodAuthority, 2017b), also indicated that practices at the retail level were unlikely to be a major contributing cause of outbreaks. However, if present, cut melons can support the growth of pathogenic bacteria. As a precaution, the NSW Food Authority recommends that vulnerable people should avoid consuming whole and pre-cut melon.

3. Risk assessment of fresh cut vegetables

In the plant products food safety scheme:

fresh cut vegetable means any of the following vegetables that has been processed in some way (for example, by trimming, cutting, slicing, peeling or pulling apart), but is still raw:

(a) capsicum,

(b) carrot,

(c) celery,

(d) leek,

(e) mushroom,

(f) spinach,

(g) chinese cabbage,

(h) cabbage,

(i) witlof,

(j) lettuce,

(k) any other leafy green vegetable.

3.1 Hazard identification – fresh-cut leafy green salad vegetables as a potentially hazardous food

The food safety scheme targets those vegetables where there is a likelihood they will be consumed raw. One of the main foods of interest in this category is leafy green salad vegetables. Numerous international risk assessments have identified leafy greens as being a high-risk fresh produce commodity. In a risk assessment by the Food and Agriculture Organization of the United Nations (FAO), leafy greens were ranked as the highest priority in terms of the safety of fresh fruit and vegetables (FAO/WHO, 2008). S*almonella*, *E. coli* O157:H7 or norovirus were identified as the pathogens of most concern for leafy greens (FAO/WHO, 2008). In the EU a risk ranking of pathogens in RTE unprocessed foods of non-animal origin, from an evaluation using outbreak data from 2007 to 2011, identified the top-ranking food/pathogen combinations. *Salmonella* spp. and leafy greens eaten raw; and norovirus and leafy greens eaten raw were identified as the third highest priority (Panel, 2013). In a semi-quantitative risk ranking tool, using data from outbreaks of confirmed aetiology that occurred in the United States, the relative public health impact of 53 pathogen-produce commodity combinations was ranked (Anderson et al., 2011). Ranking first was enterohaemorrhagic *E. coli* (EHEC) in leafy greens and ranking third was *Salmonella* spp. in leafy greens (Anderson et al., 2011).

3.2 Exposure assessment – fresh-cut leafy green salad vegetables

While there is no consumption data available for fresh-cut leafy salad vegetables in Australia, the production and consumption of minimally processed vegetables has increased worldwide (Hassenberg et al., 2017). RTE minimally processed vegetables have gained popularity due to consumer demand for fresh, convenient, preservative-free foods that may promote health. In Australia, leafy salad vegetables such as lettuce, rocket and baby spinach are the most common products in the fresh cut vegetable category (NSWFoodAuthority, 2007).

3.3 Hazard characterisation – Pathogens can grow and/or survive on leafy green salad vegetables at low temperature

3.3.1 Prevalence of pathogens on fresh-cut leafy green salad vegetables

Microbiological surveys have indicated the presence of various human pathogens on leafy salad vegetables.

The most recent targeted microbiological survey conducted by the NSW Food Authority on packaged and loose fresh cut leafy vegetables was undertaken in October 2006 (NSWFoodAuthority, 2007). The survey sampled 119 packaged and loose fresh cut leafy vegetable products from greengrocers and supermarkets around the Sydney metropolitan area. Overall, the results from the survey were excellent. When compared with the FSANZ guidelines for the microbiological examination of RTE foods 118/119 samples (99%) were categorised as satisfactory. A single sample of mixed loose-leaf salad vegetables was categorised as marginal due to the presence of low numbers of *E. coli* (4 CFU/g), just above the guideline limits of 3 CFU/g. Two other samples were found to contain very low levels of faecal coliforms (4 CFU/g). *Salmonella*, *L. monocytogenes* or verotoxigenic *E. coli* were not detected in any of the samples tested. While *L. monocytogenes* was not detected in any of the samples, the non-pathogenic *Listeria innocua* was detected in one sample of watercress. Work is currently being undertaken within the ARC Training Centre for Food Safety in the Fresh Produce Industry, to quantitatively assess the risk of human illness associated with *Salmonella* spp, *E. coli* O157:H7, and *L. monocytogenes* in locally produced leafy greens (ARCTrainingCentreforFoodSafetyintheFreshProduceIndustry, 2018b).

More recent international surveys have also been conducted. A microbiological survey was conducted in New Zealand on 307 pre-packaged fresh leafy salad products available at retail from three major cities over a one-year period (Hewitt & Rivas, 2015). *Salmonella* spp., *Campylobacter* spp., *L. monocytogenes* or Shiga toxin-producing *Escherichia coli* (STEC) were not detected in any of the samples. Nineteen samples (6.2%) were positive for other *Listeria* spp., namely *L. innocua, L. seeligeri*, *L. welshimeri, L. ivanovii* and *L. grayi*. While norovirus was detected in three (1.0%) samples.

In Canada, a survey was undertaken over four years (2009 - 2013) of 5,353 fresh-cut leafy vegetables (Denis et al., 2016). *Shigella*, *Salmonella* spp, *E. coli* O157 and *Campylobacter* were not detected in any of the fresh-cut leafy vegetables samples. The prevalence of *L. monocytogenes* (14/5,353) was 0.26%.

In the United States, a survey was undertaken over 3 years (2010 - 2012) on 14,183 RTE (i.e. samples that have been washed and processed in some manner e.g. chopped or trimmed) and bagged for retail sale, or unprocessed (i.e. samples that did not undergo further handling after harvest and before retail sale) leafy green samples (Zhang et al., 2018). The overall prevalence of *Salmonella*, *E. coli* O157:H7, non-O157 STEC, and *L. monocytogenes* for the 14,183 leafy green samples were 0.05% (7/14,183), 0.01% (2/14,183), 0.07% (10/14,183), and 0.11% (15/14,183), respectively.

In Italy, a survey revealed that 1.8% of 1160 "fresh-cut" or "RTE" leafy green vegetables retailed in supermarkets or farm markets, were contaminated with one or more foodborne pathogens harmful to human health (Losio et al., 2015). *Y. enterocolitica*, verocytotoxigenic *Escherichia coli* (VTEC) and norovirus were not detected in any of the samples. *Salmonella* spp. (6/1160), *L. monocytogenes* (4/1160) and *Campylobacter* (6/1160) were detected at a prevalence of 0.52%, 0.35% and 0.52%, respectively.

The results of microbiological surveys conducted domestically and internationally suggest that the prevalence of pathogenic bacteria in leafy green vegetables is very low. Nevertheless, the potential exists for pathogenic bacteria to be present on leafy salad vegetables.

3.3.2 Washing can't eliminate pathogens

Although on-farm good agricultural practices (GAPs) contribute to preventing pathogens entering the fresh produce chain, they cannot be relied on completely due to the open nature of farming. Postharvest processing, washing and sanitising of leafy greens is only partially effective at removing bacteria and viruses from the produce, so occasional contamination of the final product appears to be inevitable (Hewitt & Rivas, 2015). Microbial contamination present on the product may also spread throughout the production batch when the product is washed, thus increasing the risk of illnesses. The use of antimicrobials in the wash water is a critical step in preventing such water-mediated cross-contamination. Various reports have shown that pathogens such as *E. coli* O157:H7, *Salmonella* and noroviruses can be transferred from contaminated to noncontaminated vegetables in inadequately sanitised wash (Holvoet et al., 2014; Pérez-Rodríguez et al., 2014). Once contaminated, pathogens and in particular enteric viruses such as noroviruses can strongly attach and adsorb onto the leaves and persist following washing (Hewitt & Rivas, 2015). In addition, the effectiveness of washing and other intervention strategies may be compromised if pathogens are inaccessible to washing solutions or sanitisers. With produce contamination there is the potential for internalisation of pathogens, and as such the bacteria would likely not be exposed to disinfectant washes (Erickson, 2012). A recent study reported that internalisation of *Salmonella* Typhimurium and *E. coli* O157 in fresh-cut Iceberg lettuce is most likely to occur at the cut edges and wounded tissue and that of the sanitisers investigated, treatment did not result in a substantial reduction of these pathogens on the cut edges (I. Van der Linden et al., 2016).

Chlorinated agents are commonly used for water disinfection and product sanitisation. Chlorine and a number of its derivates are permitted by FSANZ as processing aids under Standard 1.3.3, at a maximum allowable concentration of free chlorine at 1.0 mg/kg. Chlorine, the most commonly used sanitiser, is primarily used to limit cross-contamination during washing operations rather than decontaminate produce *per se* (FPSC, 2015b). The use of chlorine-derived compounds to disinfect process wash water requires constant monitoring of the free chlorine and the maintenance of a residual concentration of the disinfectant in order to inhibit cross-contamination (Castro-Ibáñez, Gil, & Allende, 2017). Monitoring and maintaining an effective concentration of chlorine in commercial wash tanks can be challenging because the presence of organic matter reduces the availability of the active form of the chemical and its action is highly pH dependent (Gombas et al., 2017). A further unknown is the level of free chlorine required to achieve inactivation of the target pathogen, which will not only be dependent on the organic load and pH of the wash water, but also the temperature and produce/wash water contact time (Gombas et al., 2017). In relation to this issue, Gombas et al. (2017) have recently published guidelines to validate control of cross-contamination during washing of fresh-cut leafy vegetables.

A number of recent reviews have outlined the limitations of current post-harvest washing techniques and evaluated alternative new technologies (Meireles, Giaouris, & Simões, 2016; Ramos, Miller, Brandão, Teixeira, & Silva, 2013). While a wide variety of disinfectants other than chlorine have been evaluated, the predominance of chlorine associated with its comparatively high efficacy and low price has not been seriously challenged by any other sanitizing agent (Castro-Ibáñez et al., 2017).

3.3.3 Organisms can grow and/or survive at low temperature

The minimum temperature supporting the growth of *Salmonella* and *L. monocytogenes* on leafy greens has been reported to be 7°C and 3°C, respectively (Abhinav Mishra, Miao Guo, Robert L. Buchanan, Donald W. Schaffner, & Abani K. Pradhan, 2017). The reported minimum temperature for growth of *E. coli* O157:H7 is 6–8°C; however, in the literature it has been reported that there are instances of growth at ≤5°C, and die-off at up to 10°C (for a Review see (McKellar & Delaquis, 2011)). The ability of *L. monocytogenes* to grow at refrigeration temperatures and the potential for *E. coli* O157:H7 and *Salmonella* to grow under temperature abuse situations is therefore a concern. Juice released from the cut ends or damaged surface of salad leaves has also been reported to enhance *Salmonella* growth at refrigeration temperatures (4°C), as well as colonisation of the leaf and attachment to the salad bag plastic (Koukkidis,

Haigh, Allcock, Jordan, & Freestone, 2016). Salad leaf juices have also been reported to enhance the growth and biofilm formation of pathogenic *E. coli* (Crozier et al., 2016).

In a large study in the U.S.A, commercial time/temperature profiles were used to assess the microbial risk of *E. coli* O157:H7 and *L. monocytogenes* growth in commercially-bagged salad greens during transport, retail storage and display (Zeng et al., 2014). In the study, over a 16-month period, a series of time-temperature profiles for bagged salads were obtained from five transportation routes covering four geographic regions (432 profiles), as well as during retail storage (4,867 profiles) and display (3,799 profiles). Temperature sensors were placed in trucks during transport, in retail storage rooms, and in retail display cases in order to monitor temperature fluctuations and collect time/temperature profiles. Five different time-temperature profiles collected during 2 to 3 days of transport, 1 and 3 days of retail storage, and 3 days of retail display were then duplicated in a programmable incubator to assess *E. coli* O157:H7 and *L. monocytogenes* growth in commercial bags of romaine lettuce mix. Overall, the distribution of mean measured temperatures during transport, retail storage, and retail display, ranged from -0.3 to 7.7°C, 0.6 to 15.4°C, and -1.1 to 9.7°C, respectively. The five transport, retail storage, and retail display temperature profiles selected for the microbial studies had minimum and maximum temperature peaks ranging from 2.1 to 9.7°C, 1.8 to 18.2°C, and 1.0 to 14.1°C, respectively. In general, the data in their study reflected a commercial cold chain distribution system that was well controlled, with most temperatures under 6°C. Zheng et al. (2014) reported that temperature abuse of fresh-cut salad greens is most likely to occur during retail storage and that this aligns with the findings of others, citing studies conducted in Canada and Belgium. In the U.S.A, retail storage duration typically varies between 1 and 3 days. From their study, Zheng et al. (2014) concluded that retail storage duration has the greatest impact on pathogen growth. The short-term periods of temperature abuse simulated in their study during retail storage, led to the most significant growth of *E. coli* O157:H7 and *L. monocytogenes* (Zeng et al., 2014). While supply chain profiles may differ between the U.S.A and Australia, effective temperature control throughout the supply chain is crucial to minimising health risks associated with the potential presence of pathogens.

In addition, some fresh cut vegetables are packaged under modified atmosphere packaging (MAP) and refrigerated to extend the shelf life (NSWFoodAuthority, 2007). MAP is a food-packaging method in which the proportions of carbon dioxide, nitrogen, and oxygen in a sealed container are modified in relation to that of the normal (ambient) air to extend the food's shelf life. Next to functions such as the control of the respiration and reduction of enzymatic browning reactions, MAP conditions have also been designed to reduce the growth of spoilage microorganisms and pathogens. With additional timeframe this creates, there is an increased risk from pathogens that are able to survive and grow in these foods (NSWFoodAuthority, 2007).

While viruses cannot grow or reproduce outside a host cell, the infectious dose of norovirus is estimated to be extremely low (< 20 viral particles) (Teunis et al., 2008). Also, in contrast to bacterial pathogens, maintaining the cold-chain cannot be considered as a mitigation strategy for viral pathogens on fresh produce, as persistence of enteric viruses is higher at low temperatures (Li, Keuckelaere, & Uyttendaele, 2015).

3.3.4 History of outbreaks domestically and internationally from leafy green vegetables

In Australia, most recently there have been two outbreaks involving *Salmonella* associated with loose leaf lettuce and pre-packaged salad leaves (Appendix 1).

In the U.S.A, a study by the Centers for Disease Control and Prevention reported that between 1998 and 2008, 22.3% of all foodborne outbreaks were attributed to leafy greens (A. Mishra, M. Guo, R. L. Buchanan, D. W. Schaffner, & A. K. Pradhan, 2017). In another study, Herman et al. (2015) analysed the leafy vegetable-associated outbreaks reported to the Centers for Disease Control and Prevention between 1973 and 2012 (Herman, Hall, & Gould, 2015) and reported that there were 606 leafy vegetable-associated outbreaks, with 20,003 associated illnesses, 1,030 hospitalisations and 19 deaths. On average, leafy vegetable-associated outbreaks were larger than those attributed to other food types.

The pathogens that most often caused leafy vegetable-associated outbreaks were norovirus (55% of outbreaks with confirmed aetiology), STEC (18%) and *Salmonella* (11%).

3.4 Risk characterisation – leafy green salad vegetables as a source of food poisoning

As the potential exists for pathogenic bacteria to be present on leafy salad vegetables, there remains a possibility of sporadic cases and occasional outbreaks. Outbreaks involving leafy salad vegetables have been observed both domestically and internationally, highlighting the potential risks associated with these products.

Both outbreaks in Australia involving leafy green vegetables occurred in produce originating from Victoria (Appendix 1) and are therefore not subject to the requirements laid out in the NSW Plant Products Food Safety Scheme. In NSW the Plant Products Food Safety Scheme for fresh cut vegetables requires that *L. monocytogenes* and *Salmonella* are not detected in 25g of every ten batches tested (NSWFoodAuthority, 2016b). However, pathogenic microorganisms may be heterogeneously distributed and the number of samples from fresh-cut produce to be taken in order to be representative of a given lot/batch depends on many factors including the production system (EFSA, 2013). In addition, as can be seen from the microbiological survey data for leafy green vegetables discussed above, if pathogens are present they are likely to be present at low levels. Therefore, lack of detection of *L. monocytogenes* and *Salmonella* cannot guarantee complete absence of the pathogen.

Currently, there are no unique preventive measures or intervention strategies that could be applied at one point of the food chain to eliminate all pathogens from minimally processed leafy green vegetables. Recent reviews have emphasised the importance of a farm-to-fork approach to prevent microbial contamination of leafy greens, through strict adherence to effective GAPs, good manufacturing practices (GMPs), good hygienic practices (GHPs), and Sanitation Standard Operating Procedures (SSOPs) in primary production, postharvest handling and processing (Gil et al., 2015; Kyere, Palmer, Wargent, Fletcher, & Flint, 2018). As washing is a critical part of any RTE produce preparation, emphasis is also placed on effective monitoring and control of the sanitiser concentration, exposure time and pH. Kyere et al. (2018) also highlight the benefit of using more than one treatment system (combination treatments), which may be more effective than a single treatment. During distribution and storage, effective packaging and temperature control are essential in preventing or delaying growth of most microbes on fresh-cut leafy vegetables. These recommendations are in keeping with those of the previous Risk Assessment (NSWFoodAuthority, 2014a) in minimising health risks associated with the potential presence of pathogens. It is also recommended that the shelf-life is limited with a "use-by-date", or that the product is used quickly, so that there is not sufficient time for pathogens to grow to elevated levels (NSWFoodAuthority, 2014a).

As discussed, current manufacturing practices make it difficult to eliminate all pathogens from minimally processed leafy green vegetables. *L. monocytogenes* is a pathogen of particular concern, considering its high mortality rate and that the incidence of systemic listeriosis is much higher in susceptible populations. The NSW Food Authority has included an additional control measure for packaged pre-cut vegetables in relation to *L. monocytogenes* and vulnerable persons, by limiting the shelf life to no more than 7 days from the date of packaging (NSWFoodAuthority, 2018b). The NSW Agency for Clinical Innovation has developed a low microbial diet (ACI, 2017) that may be considered on a case-by-case basis by consulting medical staff and dieticians in choosing whether to exclude leafy green vegetables from the diet of severely immunocompromised patients.

4. Risk assessment of seed sprouts

In the Plant products food safety scheme, seed sprouts are defined as:

"sprouted seeds (other than wheat grass) or sprouted beans."

4.1 Hazard identification – *Salmonella* and Shiga-toxin producing *E. coli*

Seed sprouts have been implicated in food-borne illness outbreaks both domestically and internationally. These outbreaks have been associated with various types of sprouts contaminated with a variety of pathogens, including *Salmonella*, *Escherichia coli* O157:H7 and other Shiga-toxin producing *E. coli*. Other bacterial pathogens (e.g. *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Yersinia enterocolitica*) have also been implicated in sprout-associated outbreaks, although these have been reported very rarely (EFSA, 2011). In a through-chain analysis of food safety hazards and control measures associated with the production and supply of seed sprouts, FSANZ reported that the key pathogenic microorganisms of concern are *Salmonella* spp. and STEC (H. Jin et al., 2014).

In Australia, of the 7 sprout-related foodborne outbreaks between 2011 and 2016 (FSANZ, 2018a), the most frequently reported aetiological agent was *E. coli* (4), followed by *Salmonella* (3). In the U.S.A, of the 56 sprout-related outbreaks that occurred between 1998 and 2016; for which an aetiological agent was reported (CDC, 2018b), the vast majority were caused by *Salmonella enterica* (39), followed by Shiga toxin-producing *E. coli* (10), *Listeria monocytogenes* (3) and norovirus (1). In Europe, more than 50 sprout-associated outbreaks were reported over the last 20 years and *Salmonella* and Shiga toxin-producing *E. coli* were the most frequent causative agents (EFSA, 2011; Margot, Ebner, Peterhans, & Stephan, 2016).

While *Salmonella* has been the primary pathogen involved in the majority of sprout-associated outbreaks globally, Shiga toxin-producing *E. coli* pose a particular hazard due to their low infectious dose and the potentially severe consequences of infection. Shiga toxin-producing *E. coli* strains can cause infections that can range from mild diarrhoea to the potentially fatal haemolytic uremic syndrome (HUS). The term EHEC is used to designate a subset of Shiga toxin–producing *E. coli* that cause severe diseases in humans, including bloody diarrhoea and HUS. One of the largest outbreaks to date of EHEC was recorded in Germany in 2011 and involved contaminated fenugreek seeded sprouts. In this outbreak, 3,842 people became ill, of which 2,987 had acute gastroenteritis, 855 suffered from HUS and 53 people died (35 HUS patients and 18 with gastroenteritis) (Appel, Böl, Greiner, Lahrssen-Wiederholt, & Hensel, 2012).

In Australia, the majority of FSANZ recalls between 2011 and 2016 due to biological contamination of sprouts were due to mixed sprouts (4), followed by mung bean sprouts (3) (FSANZ, 2018a). In the U.S.A, from 1996 to 2016, the FDA was involved in 48 investigations involving sprout-associated outbreaks. The majority of outbreaks were attributed to alfalfa sprouts (30), followed by clover (7), mung bean (6) and sprouted chia powder (1) (Gensheimer & Gubernot, 2016). In Europe, alfalfa and mung bean sprouts, which are the most commonly consumed types of sprouts, were most frequently associated with outbreaks (EFSA, 2011; Margot et al., 2016).

4.2 Exposure assessment

Consumption of sprouts – sprouts are not a widely consumed product

The number of licensed processors in NSW remains small, with 8 facilities licensed to process seed sprouts in the state. Sprouts are generally consumed because of health and culinary factors (e.g. the use of bean sprouts in culinary dishes) and where they are incorporated in dishes as a garnish (FSANZ, 2009).

Consumption data for sprouts from the 1995 NNS and the 2011-2012 NNPAS are shown in Table 3. Data for sprouts from the 1995 NNS and 2011-2012 NNPAS includes consumption of alfalfa, bean and snow pea sprouts. In both surveys data for sprout consumption also captures sprouts included in mixed dishes (e.g. fried rice, salads, soups,

spring rolls and stir-fries). Mean consumption results are provided for the number of consumers, as well as the proportion of consumers out of the total respondents in the population group (see Appendix 4 for respondent numbers in each population group assessed).

Sex	Age group (years)	Number of consumers		% consumers		Consumer mean consumption (g/day)		Respondent mean consumption (g/day)	
		1995 NNS	2011-2012 NNPAS	1995 NNS	2011-2012 NNPAS	1995 NNS	2011-2012 NNPAS	1995 NNS	2011-2012 NNPAS
Females	2+	567	131	7.8	2.1	18	21	1.0	0.5
	2-5	13	NC	3.1	0	8	0	0.3	0
	6-12	23	NP	3.7	NP	14	NP	0.5	NP
	13-18	34	NP	7.8	NP	25	NP	2.0	NP
	19+	497	115	8.6	2.4	18	22	1.5	0.5
Males	2+	530	107	8.0	1.8	21	39	2.0	0.7
	2-5	20	10	5.3	3.0	7	11	0.3	0.3
	6-12	46	NP	6.9	NP	15	NP	1.0	NP
	13-18	34	NP	6.9	NP	18	NP	1.3	NP
	19+	430	90	8.5	1.9	22	44	1.9	0.8
Persons	2+	1097	238	7.9	2.0	19	29	2.0	0.6
	2-5	33	10	4.2	1.6	7	11	0.3	0.2
	6-12	69	13	5.4	1.1	15	15	0.8	0.2
	13-18	68	10	7.3	1.2	22	18	1.6	0.2
	19+	927	204	8.5	2.2	20	32	1.7	0.7

*Based on day 1 of the nutrition survey only. Includes sprouts in mixed foods/dishes.

NC = not consumed

NP = not publishable, too few consumers

The average quantities of sprouts consumed by all age groups in both the 1995–1996 and 2011–2012 surveys was small (Table 3). For reasons outlined previously, care should be taken when comparing data between surveys (FSANZ, 2018b). In absolute terms, the total mean consumer consumption (g/day) of sprouts was higher in the 2011-2012 NNPAS. While the total mean respondent consumption (g/day) of sprouts was higher in the 1995 NNS.

4.2.2 Prevalence of hazards in sprouts

The NSW Food Authority conducted a survey between March 2015 and December 2016, in which 113 sprout samples were tested for *Campylobacter*, *Salmonella*, *E. coli* and a SPC was performed (NSWFoodAuthority, 2018a). Two samples of mung bean sprouts contained *Salmonella*, with both samples taken from the same producer over a short time period. Five samples contained *E. coli* at a level above >10 CFU/g, which was not unexpected as the samples were raw produce. However, *E. coli* was detected at unexpected elevated levels (above 3,000 CFU/g) in two of these

samples, and *Campylobacter* was detected in one sample (quantification was not conducted). The NSW Food Authority also conducted a survey on the microbiological quality of sprouts in NSW in 2008 (NSWFoodAuthority, 2008). A total of 122 sprout samples, representing 99.9% of all sprouts produced in NSW, were tested over a three month period. Samples included alfalfa, mung bean and 'others' such as radish, onion and broccoli. No *E. coli* (including VTEC), *Salmonella* or *L. monocytogenes* were detected in any samples. Results were compared to "Guidelines for the microbiological examination of ready-to-eat foods" (FSANZ, 2001). Of those samples tested, 99.2% were classified as microbiologically acceptable. All samples that were rated marginal and unsatisfactory were due to *B. cereus*, which was detected at unsatisfactory levels in one sample (5500 CFU/g) and at the limit of detection (100 CFU/g), classified marginal, in four samples. Since this survey was conducted, a new standard in the Australia New Zealand Food Standards Code came into effect in July 2013, requiring a decontamination step prior to sale or supply of seed sprouts (FSANZ, 2012b).

More recent microbiological surveys have been conducted outside of NSW. A microbiological survey was also conducted in the ACT in 2014 (Waters, Krsteski, & Rockliff, 2014), to look at the microbiological quality of 57 samples (12 snow pea sprouts and 45 seed sprouts) from 11 ACT retail outlets. The microbiological quality of the seed sprouts and snow pea sprouts was assessed based on the FSANZ Guidelines for the microbiological examination of RTE foods (FSANZ, 2001) *Salmonella* and *L. monocytogenes* were not detected in any of the samples. However, marginal levels (3-100 CFU/g) of generic *E. coli* were found in three samples of seed sprouts (6.38%). A survey was also undertaken in Victoria in 2015 (Symes, Goldsmith, & Haines, 2015) to detect specific microbes in sprout products (bean shoots, alfalfa, bean sprouts, snow pea sprouts, mixed products and mung bean sprouts) as sold and used by small businesses, including wholesale and retail outlets and food preparation premises. A total of 298 seed sprout samples were collected from across 33 local council areas. The samples were also assessed against the RTE microbiological guidelines set by FSANZ (FSANZ, 2001). In the sprout products tested, generic *E. coli* was detected at 100 CFU/g (deemed unsatisfactory) in 3.7% of samples and between 3 and 100 CFU/g (deemed marginal) in 11.1% of samples. *L. monocytogenes* was detected in 1.4% of samples at a marginal level (<100 CFU/g). *Salmonella* spp. was not detected in any of the samples.

Comparison of the results of the Australian microbiological surveys is complicated by differences in sampling plans, microorganisms targeted and a lack of equivalence between detection methods. However, *Salmonella* was not detected in any of the most recent surveys conducted in NSW, the ACT or VIC. *L. monocytogenes* was not detected in NSW or the ACT, but was present at marginal levels in VIC. While *E. coli* was not detected in any of the NSW samples, generic *E. coli* was detected at marginal levels in the ACT and at marginal and unsatisfactory levels in VIC. The lack of detection of *E. coli* in NSW samples, has been attributed to the revision of sprout safety requirements for sprout producers introduced in 2007 (NSWFoodAuthority, 2008).

A number of microbiological surveys have also recently been conducted overseas. In New Zealand, a microbiological survey was conducted in 2014 of RTE packaged seed sprouts and shoots, available at supermarkets, independent sellers and farmers markets (D'Sa & Paulin, 2015). Fifty different batches of seed sprouts and shoots were purchased, and the samples were analysed for levels of *E. coli* and mesophilic aerobic microflora. Five units of each of the 50 batches of seed sprouts and shoots were mixed to form a composite sample and were analysed for the presence of *Salmonella* spp., *Listeria* spp., *L. monocytogenes* and STEC. When any of these microorganisms were detected, the individual subsamples were further tested to enumerate the concentrations of *Listeria* spp., *L. monocytogenes* and *Salmonella* spp. *L. monocytogenes* was detected in a single composite sample of a batch of sunflower seed sprouts at levels <100 CFU/g. *Listeria* spp. (excluding *L. monocytogenes*) was detected in six composite samples (6/50) of seed sprouts and shoots at levels <100 CFU/g. STEC was not detected in any of the composite samples and the concentration of generic *E. coli* in the majority of individual subsamples (218/222) was <3 MPN/g. *S.* Adelaide was detected in 2 out of 5 individual subsamples from 1 composite sample of alfalfa sprouts and snow pea shoots with

counts of 0.04 MPN/g. The detection of *L. monocytogenes* and *S.* Adelaide in a small number of individual subsamples and composite samples, it was evident that some RTE seed sprouts and shoots available in New Zealand do not conform with the microbiological limits set out in the Australia New Zealand Food Standards code 1.6.1.

In the U.S.A., the FDA set out to collect and test domestically grown sprouts between 2014 and 2016 (FDA, 2017b). The prevalence of *Salmonella*, *L. monocytogenes* and *E. coli* O157:H7 in sprouts was determined through testing of a total of 1,600 samples of seeds, finished product and spent irrigation water. The FDA collected samples of 14 different varieties of sprouts in total. The most frequently collected were mung bean (36%), alfalfa (21%), clover (10.4%) and soybean (10.4%). Other sprout varieties sampled included adzuki bean, broccoli, kale, mustard seed, onion, pea, radish, snow pea, sunflower and wheat. In addition, 5% of the samples collected were described as "mixes," meaning they contained two or more varieties. Though all samples were collected in the United States, 72 of the 170 seed samples were of international origin (from Australia, Canada, China and Italy). The prevalence of *Salmonella* in seeds (2.35%) was significantly higher than in finished product (0.21%) and in spent irrigation water (0.54%). The prevalence of *L. monocytogenes* in the finished product was 1.28% and there was no significant difference in the prevalence of *L. monocytogenes* at any of the other points in the production process (seeds, 0.59%; spent irrigation water, 0.54%). None of the samples tested positive for *E. coli* O157:H7. The agency did not test seed for *E. coli* O157:H7 due to limitations associated with the test method. The results of the survey led to the conclusion that seeds for sprouting can be an important source of *Salmonella* contamination and that the production environment can be an important source of *Listeria monocytogenes* contamination.

4.3 Hazard characterisation – Sprouts produced in NSW have been linked to recent foodborne outbreaks

Since the last risk assessment (NSW Food Authority, 2014a), there have been four foodborne outbreaks linked to sprouts in Australia (Appendix 1). Of these four outbreaks, three were linked to sprout producers in NSW. Detailed information on these outbreaks is limited to that of the 2016 outbreak, outlined in the OzFoodNet NSW Annual Report 2016 (Communicable Diseases Branch, 2017). A multi-state outbreak of *Salmonella* Saintpaul was linked to mung bean sprouts. In NSW, 99 confirmed and 17 probable cases were linked to the outbreak with specimen collection dates between December 2015 and April 2016. Health advisories and product recalls occurred in jurisdictions where the implicated product was distributed.

Mung bean sprouts were also linked to an outbreak involving *Salmonella* Saintpaul in sprouts originating in South Australia and distributed in SA, VIC and NT. The source of the issue was traced to contaminated mung bean seeds from a supplier in QLD. It should be noted that the SA sprout producer was complying with the FSANZ Sprout standard, which does not include a seed decontamination step of a soak in 20,000ppm or stronger solution of Calcium hypochlorite (or another sanitiser solution of equivalent effectiveness), which is specified in guidance documents by the NSW Food Authority (NSW Food Authority, 2005).

4.4 Risk characterisation – no validated critical control point (CCP) exists

Seed sprouts remain a high risk food as the seeds are germinated and grown under conditions (time, temperature, water activity, pH and available nutrients) that are conducive to the growth of pathogens and there is no validated Critical Control Point (CCP) which prevents, eliminates or reduces the pathogen to an acceptable level (Kiermeier, May, & Holds, 2013). Foodborne pathogens, such as *Salmonella* and *E. coli*, may multiply by several logs on the sprouting seeds during their short growing period (D., K., J., T., & M., 2001; Stewart, Reineke, Ulaszek, & Tortorello, 2001). The lack of a validated CCP for faecal pathogens such as *Salmonella* and pathogenic *E. coli* imposes significant risk on the sprouting process (Kiermeier et al., 2013).

4.4.1 Seed decontamination

Although various routes of contamination have been noted, contaminated seeds appear to be the source of most sprout-associated foodborne illnesses and are considered the most common source of contamination. Seed contamination may come from the soil, pests, irrigation water and processing equipment. Research indicates that seed contamination, when it occurs, may be at low levels, intermittent, or unequally distributed within seed lots (FDA, 2017a). However, even low levels of human pathogens on seed for sprouting are a concern, due to the ideal growth conditions present during sprouting. While treating seeds used for sprouting does not guarantee pathogen-free sprouts, seed treatment reduces the percentage of contaminated batches. Therefore, seed treatment is a critical part of a multi-hurdle approach to reduce the public health risks associated with sprouts.

The Primary Production and Processing (PPP) Standard for seed sprouts (Standard 4.2.6) issued by FSANZ includes the need to 'implement effective decontamination processes prior to sale or supply of seed sprouts' but does not stipulate a microbial reduction that needs to be achieved. The Standard allows the use of any scientifically valid method to reduce microorganisms of public health significance on seeds used to grow sprouts. This approach provides flexibility in choosing a seed treatment. The validity and efficacy of any treatment is dependent on the specific parameters used, e.g., seed type, treatment concentration, treatment time, temperature, etc. In addition, the practicality of any decontamination step is determined by a number of factors, such as the lethal effect on pathogens, rate of loss of germination and the safety using the chemicals for decontamination (NSWFoodAuthority, 2014a). In the previous Risk Assessment (NSWFoodAuthority, 2014a), the only potential decontamination treatments deemed practical for the Australian industry were calcium hypochlorite, peractic acid and dry heat. Treatment with high concentrations of free chlorine remains a common seed decontamination practice and calcium hypochlorite is the most commonly used chemical decontaminant used by industry (Kiermeier et al., 2013).

In the U.S.A., 20,000 ppm of calcium hypochlorite is cited in the FDA's Guidance document for the sprout industry for seed treatment (FDA, 2017a). However, its implementation in commercial sprouting facilities has several disadvantages, including the potential hazard to workers and the environment with the high level of chlorine (Ding & Fu, 2016). In addition, the effectiveness of calcium hypochlorite (20,000 ppm) as a seed decontamination treatment has been questioned, as outbreaks involving *S.* Typhimurium (Brooks et al., 2001), *S.* Muenchen (Proctor, Hamacher, Tortorello, Archer, & Davis, 2001) and *E. coli* O157:NM (Ferguson et al., 2005) have occurred from batches of seeds reportedly treated following FDA seed decontamination guidelines. Additional seed treatment methods that are effective and can be applied safely and consistently during commercial sprout productions are needed.

Recently, the FDA reviewed current seed treatment methods, including those that work by chemical means (liquid or gas), physical means, or a combination of these methods applied sequentially or simultaneously (FDA, 2017a). Physical and combination style treatments were reported to be the most effective for removing pathogens from seeds for sprouting. Physical treatments, such as heat (dry heat or hot water) and high pressure are reported to have better penetration characteristics for reaching bacteria sheltered in scarified surfaces and the interior of the seeds as compared to chemical treatments (Ding, Fu, & Smith, 2013). Physical treatments such as high pressure (Neetoo, Ye, & Chen, 2008) and combination methods such as dry heat and sanitiser (Bari, Nei, Enomoto, Todoriki, & Kawamoto, 2009) or dry heat and high pressure (Neetoo & Chen, 2011), have been reported to achieve a 5-log or greater reduction in pathogens on seeds. An important consideration in assessing the suitability of seed treatment methods is whether they have a negative impact on seed germination. Neetoo et al. (2008) demonstrated that high-pressure processing (HPP) treatment of alfalfa seeds for 15 minutes at 650 MPa and 20°C, achieved a 5-log reduction in *E. coli* O157:H7 and that pressure-treated seeds achieved a germination rate identical to untreated seeds after eight days of sprouting. Bari et al. (2009) demonstrated that broccoli and alfalfa seeds treated with dry heat at 50°C for 17 hours, followed by soaking in either 1% oxalic acid, 0.03% phytic acid, 50% ethanol, electrolysed acidic water or electrolysed alkaline water solutions, resulted in a 5-log reduction in *E. coli* O157:H7. In addition, Bari et al. (2009) reported that

irrespective of treatment conditions, no significant difference was observed in percent germination of the finished sprouts. Neetoo and Chen (2011) reported that a dry heating step (60°C for 24 hours) in combination with high pressure (600 MPa for 2 minutes at 35°C) led to a 5-log reduction in *Salmonella* and *E. coli* O157:H7 on alfalfa seeds. While this sequential dry heat and HPP treatment did not significantly affect the germination percentage of alfalfa seeds, a >20% reduction in the sprout yield was observed (Neetoo & Chen, 2011). Further studies are warranted to determine whether the sprout yield could be improved for seeds subjected to dry heating and high pressure, if a restoration step were included during seed storage prior to germination.

As current seed treatment technologies are unlikely to ensure complete elimination of microbial contamination of seeds and sprouts, sampling and testing programs are critical complementary intervention strategies.

4.4.2 Sampling and analysis requirements for seed sprout processors in NSW

In contrast to other states and territories in Australia, in NSW the NSW Food Safety Schemes Manual (NSW Food Authority, 2016b) requires seed sprout processors to test spent water and the final product (Table 4). The testing required may allow sprout producers to detect any microbiological issues before the sprouts reach the market.

In addition, microbiological limits contained in the Australia New Zealand Food Standards Code (FSANZ, 2017), also specify that for cultured seeds and grains, no *Salmonella* species should be detected in 25g for any samples of final product analysed. Furthermore, as seed sprouts and shoots are a RTE product in which the growth of *L. monocytogenes* can occur (Farber, Carter, Varughese, Ashton, & Ewan, 1990; Schoeller, Ingham, & Ingham, 2002), *L. monocytogenes* must not be detected in 25g for any samples of final product analysed.

Table 3: Sampling and analysis requirements in NSW for seed sprout processors

Product to be tested	Test to be conducted	Limit	Frequency
Seed used for sprouting (pre-screening test)	Salmonella Method: 1L sample of spent irrigation water from a test bath of seeds made up of 3kg taken evenly across the batch	Not detected in 100 mL	Every delivery batch of seeds
Spent irrigation water used for seed sprouting	Salmonella Method: 1L composite sample taken evenly across each sprouting container from each production batch. Irrigation water should be sampled just before harvest or at least 48 hrs after lay.	Not detected in 100 mL	Every 10 batches
Seed sprouts (finished product)	E. coli Method: 1 x 100g sample of any finished single sprout-type from each process line	Not exceeding 100 /g	Every 10 batches

While sampling and analysis requirements are mandated for seed sprout processors in NSW (NSWFoodAuthority, 2016b), sprout-associated outbreaks originating in NSW have continued to occur (Appendix 1). Because microbial contamination in seeds, if present, is often at low population levels and not uniformly distributed throughout a seed lot, such contamination may not necessarily be detected through sampling under the NSW Food Safety Schemes Manual (NSW Food Authority, 2016b).

Overall this updated seed sprout risk assessment is based on previous risk assessment work and documents. It supports the use of food safety programs to control risks. As current seed decontamination methods can reduce but not eliminate pathogens, novel seed production and processing methods appropriate for use by conventional and organic producers are needed to improve the microbial safety of sprouts.

5. Risk assessment of vegetables in oil

The Plant products food safety scheme defines vegetables in oil as:

a) fruits, vegetables or herbs, or

b) parts of fruits, vegetables or herbs, or

c) a combination of anything in paragraph (a) or (b),

immersed wholly or partly in oil.

Standard 2.3.1 of the Code requires that fruit and vegetables in brine, oil, vinegar or water must not have a pH greater than 4.6.

5.1 Hazard identification – botulism is a potential risk

The potential for foodborne botulism from vegetables in oil products is an ongoing concern, particularly for poorly acidified products (NSW Food Authority, 2011, 2014a). While these products are safe if refrigerated correctly during distribution at the retail level and by the consumer, they represent a potential food poisoning hazard unless certain basic precautions are taken in their preservation (NSW Food Authority, 2011). There is an inherent risk in the processing of these products if they are not prepared appropriately. Botulism is caused by the neurotoxin formed by the anaerobic, spore-forming microorganism *Clostridium botulinum*. Spores formed by *C. botulinum* are ubiquitous in the environment and might be found on the surfaces of fruits and vegetables. To prevent foodborne botulism, it is necessary to identify and apply control measures that destroy spores, or prevent spore germination, cell multiplication and neurotoxin formation. Foods with a pH below 4.6 do not in general support the growth of food poisoning bacteria, including *C. botulinum.* As *C. botulinum* will not grow in acidic conditions (pH less than 4.6), the toxin will not be formed in acidic foods (however, a low pH will not degrade any pre-formed toxin) (WHO, 2018).

Attempts to preserve these products without acidification seem to be based on two false assumptions: 1) that the addition of oil has a preservative effect and, 2) that some herbs and spices, and especially garlic, have significant anti-microbial properties (CSIRO, 2017). Oil does not have a preservative effect. The only function of the oil is to prevent oxidation from the air in the container, which can lead to discolouration of some foods. By excluding air from the surface of the food, oil establishes an anaerobic environment that favours the growth of *C. botulinum.* Addition of herbs, spices and garlic will not have a preservative effect that guarantees the safety of these products. It is therefore essential that sufficient acid is added to the vegetable before oil is poured on so that any *C. botulinum* or other potentially dangerous bacteria cannot grow. This will not guarantee that the products will not spoil if not kept properly refrigerated, but it will ensure they do not become potentially hazardous.

If the vegetables are dried prior to being stored in oil, a different set of circumstances applies. Correctly dried vegetables and herbs will not support the growth of food poisoning bacteria, but they may still support the growth of spoilage organisms such as yeasts and moulds. Moulds will usually only be a problem on exposed surfaces but yeasts bring about fermentation in the absence of air (CSIRO, 2017).

5.2 Exposure assessment – vegetables in oil remain a niche product

The number of commercial manufacturers of these niche products remains small, with only 8 businesses licensed in NSW. The increasing popularity of farmers markets may encourage people to attempt to manufacture these products without the skills and knowledge necessary to produce a safe product.

5.3 Hazard characterisation – severe outcomes rare but possible

The botulinum neurotoxins are the most potent poisons known, and foodborne botulism may be caused by consuming as little as 50 ng of neurotoxin (Michael W. Peck & van Vliet, 2016). Botulinum toxins block nerve functions and can lead to respiratory and muscular paralysis. While botulism poisoning is rare, because of its severe, debilitating

symptoms and relatively high mortality rate of approximately 5–10% of cases (M.W Peck, 2006), it remains a major hazard in products produced by commercial businesses and in the home. There have been no cases of foodborne illness attributed to these products in Australia. However, a failure to apply suitable control measures could lead to outbreaks of foodborne botulism, as occurred in the 1980's with inadequately acidified commercial garlic-in-oil products in Canada (St. Louis, Peck, Bowering, & et al., 1988) and the USA (Morse, Pickard, Guzewich, Devine, & Shayegani, 1990). In 1991 Australian authorities regulated within Standard 2.3.1 of the Code that this class of product must not have a pH greater than 4.6.

5.4 Risk characterisation – regulations still applicable to manage risk

The application of food safety skills and knowledge is very important for these products to be made safely. As outlined above, issues have arisen when home made products are attempted to be made in commercial quantities. The requirements of the food safety scheme provide a baseline entry level to manufacture these products commercially and may be a deterrent to those without the necessary skills to make these products.

6. Risk assessment of unpasteurised juices

The Plant products food safety scheme defines unpasteurised juice as:

"fruit or vegetable juice, or a mixture of such juice, that has not been subject to pasteurisation." Pasteurisation, in relation to fruit or vegetable juice, is defined as heating the juice to a minimum temperature of 72°C for 15 seconds, or treating the juice using a technology or method that produces an equivalent lethal effect on microorganisms present in the juice.

6.1 Hazard identification – pathogenic *E. coli* and *Salmonella* spp.

Globally, the pathogens that have been responsible for the majority of outbreaks in unpasteurised juice are *E. coli* O157:H7 and *Salmonella* (Jackson-Davis et al., 2018). The acidity of fruit juice has historically been thought to be an important barrier against survival and growth of foodborne pathogens (Jackson-Davis et al., 2018). At a pH value of 4.5 or less, the acidic conditions effectively reduce the risk of growth of pathogenic organisms (M.E. Parish et al., 2003). However, care needs to be exercised with products that have pH values that are within the range of 3.9–4.5, as it has been demonstrated that some pathogens can survive under such conditions (Ashurst, 2011). For example, *E. coli* O157:H7 was shown to survive in apple cider (pH 3.56 to 3.98) and orange juice (pH 3.82 to 3.86) held at 5 or 25°C for up to 42 days (Ryu & Beuchat, 1998). *Salmonella* was also reported to maintain the same population level in orange juice at refrigeration temperature at pH 3.5, 3.8, 4.1, and 4.4 for 5, 10, 15, and 20 days, respectively (M. E. Parish, 1997). In addition, the higher pH of some fruit (e.g. melon) and all vegetable juices, would not inhibit the growth of pathogens. For many non-traditional juice products, where outbreaks have not previously occurred, there is limited information available for producers to determine the appropriate pathogen of concern (Jackson-Davis et al., 2018).

6.2 Exposure assessment – low levels of production

There are 12 licensed facilities making unpasteurised juice in NSW, with another 6 businesses having the approval to manufacture juice as a secondary approved activity. At the time of the previous risk assessment (NSWFoodAuthority, 2014a), there were six licensed facilities making unpasteurised juice in NSW, with another two businesses having the approval to manufacture juice as a secondary approved activity.

The scope of the scheme does not extend to unpasteurised juice produced at retail establishments. These retail juice bars come under the jurisdiction of local councils. Unpasteurised fruit juice has gained considerable popularity mainly due to the demand for foods that have not been subjected to processing methods (such as heat) that may lead to loss of colour, flavour, vitamins, and minerals (Jackson-Davis et al., 2018). Therefore, the popularity of these outlets has increased, but it is unclear what proportion of unpasteurised juice consumed by the population originates from retail outlets. With this increased popularity, there is also an increased risk of foodborne illness.

6.3 Hazard characterisation – no recent outbreaks

There have been no recent outbreaks in Australia involving unpasteurised juice. The last outbreak in Australia occurred in South Australia in 1999, when over 500 customers becoming ill after consuming Nippy's orange juice contaminated with *Salmonella*. This outbreak was traced back to oranges from a fresh fruit packing house. *Salmonella* was traced to the wash tank where fungicide was added to the oranges, but it was not clear where the initial contamination originated. This outbreak led to the introduction of a requirement for citrus packers in South Australia to have a food safety program.

6.4 Risk characterisation – new technology as an alterative

The risk from unpasteurised juice is relatively low, provided good quality produce is used to manufacture the juice and that the produce is washed and/or sanitised prior to juicing. It is considered that the implementation of a food safety program and verification testing of finished product is sufficient to manage the risk. The NSW Food Safety Schemes

Manual (NSWFoodAuthority, 2016b) requires unpasteurised juice processors to conduct final product testing. The Manual mandates that no *Salmonella* species should be detected in 25g for any samples of final product analysed. As outlined in the Industry Guide for the Development of a Food Safety Program (High Priority Plant Products Industry) (NSWFoodAuthority, 2005), another important element of managing risk is maintaining temperature control (≤5°C) during storage and distribution of product. Botulism illnesses associated with refrigerated carrot juice occurred in Canada and America in 2006 (CDC, 2006; Sheth et al., 2008) and led to the FDA modifying its guidance for refrigerated low-acid juices to recommend adding a validated juice-treatment method, such as acidification to a pH of 4.6 or below or appropriate thermal treatment, to decrease the risk of *C. botulinum* growth and toxin formation, should any breaches in refrigeration occur (FDA, 2007). The FDA also recommends that firms continue to utilise a label statement such as "Keep Refrigerated" (FDA, 2007). In Australia, Application A411 to the Australia New Zealand Food Authority (ANZFA) in 2001 to require unpasteurised juice to be labelled – indicating that the juice was not pasteurised, did not proceed (ANZFA, 2001).

There has been a growing interest in the design of novel processing technologies that can be used in place of thermal pasteurisation to inactivate microbial pathogens without affecting sensory and nutritional properties during commercial juice processing (Bevilacqua et al., 2018; Jiménez-Sánchez, Lozano-Sánchez, Segura-Carretero, & Fernández-Gutiérrez, 2017). However, more detailed studies are needed on the scaling-up, process design, and optimisation, as well as on the effect of such technologies on juice quality in order to maximise their potential as alternative nonthermal technologies in juice processing (Jiménez-Sánchez et al., 2017). High-pressure processing (HPP) is the most developed and most widely embedded technology at the industrial level (Bevilacqua et al., 2018). Products made using HPP technology which delivers an equivalent lethality to heat pasteurising, are considered pasteurised and manufacturers do not require a license.

7. Chemicals in plant products

Standard 1.4.2 of the Code lists the maximum residue limit (MRL) for agricultural and veterinary chemical residues which may occur in foods in Australia. Limits prescribed in the Code constitute a mandatory requirement applying to all food products of a particular class whether produced domestically or imported.

Noncompliant residue results above the MRL very rarely equate to food safety risks, but can lead to reviews to identify potential areas of improvement in agricultural practices (MPI, 2017). In 2019, the NSW Food Authority will conduct a survey of produce to further inform risk assessments and the identification of potential areas for improvement in the production of these commodities. The NSW Food Authority recommends that all fruit and vegetables are washed with cool tap water immediately before eating (NSWFoodAuthority, 2015a).

An emerging issue also warranting discussion, is the link between the use of agricultural azole-based fungicides and the selection pressure for azole resistant strains of *Aspergillus fumigatus* (J. W. M. van der Linden et al., 2015; Jan W. M. van der Linden et al., 2011; Verweij, Snelders, Kema, Mellado, & Melchers, 2009). Worldwide, azoles are the dominant chemicals used in the treatment of fungal infections in crops, humans and livestock (Fisher, Hawkins, Sanglard, & Gurr, 2018). Multi-azole–resistant *A. fumigatus* has been recovered from environmental and clinical samples globally (Fisher et al., 2018). Azoles are increasingly failing as frontline therapies, with associated patient mortality approaching 100% (van Paassen, Russcher, In 't Veld-van Wingerden, Verweij, & Kuijper, 2016). The prevalence of azole resistant strains of *A. fumigatus* varies with geographical region and is highest in Europe (3%–26%) (Chowdhary, Sharma, & Meis, 2017; Garcia-Rubio, Cuenca-Estrella, & Mellado, 2017; J. W. M. van der Linden et al., 2015), where azoles total more than 26% of all fungicides used in crop protection (EuropeanCentreforDiseasePreventionandControl, 2013). A recent study concluded that azole resistance is uncommon in Australian clinical and environmental *A. fumigatus* isolates; but that further surveillance is warranted (Talbot et al., 2018). In addition to increased surveillance of antifungal resistance, research is required into alternatives to antifungal drugs and further consideration as to how to minimise resistance developing from their use.

8. Horticultural products outside of the scope of the Safety Scheme

Since the last Plant Products Risk Assessment (NSWFoodAuthority, 2014a) there have been a number of outbreaks linked to products outside of the current scope of the Plant Products Food Safety Scheme. There have been two recent outbreaks in Australia involving whole rockmelons (Appendix 2). The scope of the Plant Products Food Safety Scheme does not include whole raw fruits and vegetables, which are not intended to be further processed. In the last Plant Products Risk Assessment (NSWFoodAuthority, 2014a), whole rockmelons were also discussed as an out of scope product in light of a listeriosis outbreak that occurred in 2010. There have also been four outbreaks of hepatitis A in Australia that have been linked to imported frozen berries (Appendix 3). The scope of the Plant Products Food Safety Scheme does not include imported products, which fall under the remit of the Department of Agriculture and Water Resources (DAWR). However, the recent recalls of imported berry products highlight the potential for food safety issues in domestically produced berries. Therefore, whole rockmelons and berries are discussed below, in addition to a number of other products identified during the environmental scan as potentially high-risk products. These additional products include fermented nut cheeses, plant-based meat alternatives and microgreens.

Previously, the NSW Food Authority identified a number of additional plant-based products as potentially "high risk" (NSWFoodAuthority, 2014b). The products identified included tofu, tempeh, kimchi, vegetable-based dips, mixed salads, fresh herbs and edible seaweed (NSWFoodAuthority, 2014b). A survey was conducted to determine the microbiological quality and/or chemical properties of these products (excluding kimchi and seaweed) and overall the food safety issues concerning these products were considered to be rare and sporadic. Based on this assessment, it was concluded that requirements set out in the Food Standards Code coupled with inspection of businesses are likely to provide adequate food safety control. Further regulation by broadening the scope of the plant products food safety scheme was deemed not to be warranted. There have been no foodborne outbreaks within Australia linked to these products since the review was undertaken, indicating that these recommendations remain valid.

8.1 Whole rockmelons

Since the last Plant Products Risk Assessment (NSWFoodAuthority, 2014a), there have been two outbreaks in Australia involving whole rockmelons. *Salmonella* was linked to an outbreak in 2016 and more recently, *L. monocytogenes* was linked to an outbreak in 2018 (Appendix 2). The 2018 listeriosis outbreak resulted in 22 confirmed cases, including 7 deaths and 1 miscarriage (NSWDPI, 2018). The average age of those affected was 70, with a range of 0-94 years (0 years - live birth at 36 weeks) (NSWDPI, 2018). The scope of the Plant Products Food Safety Scheme does not include whole raw fruits and vegetables, which are not intended to be further processed. Therefore, the current scheme does not extend to on farm practices or to packing sheds where the contamination may have occurred. The recent occurrence of outbreaks involving rockmelons indicates that additional regulatory or non-regulatory measures are warranted to see improved food safety outcomes within this sector in conjunction with existing schemes.

Salmonella and *L. monocytogenes* are the two pathogens that have been most often associated with foodborne illness outbreaks from melons domestically and internationally. *Salmonella* and *L. monocytogenes* are ubiquitous in nature and can persist in the soil, even under harsh environmental conditions, for long periods of time (Islam et al., 2004; Strawn et al., 2013). Rockmelons are grown at ground level and their outer skins can be contaminated with pathogenic bacteria during production from irrigation water and manure fertilisers, and during food processing by contaminated process water, equipment and food handlers. The most probable sources for biological contamination of melons during primary production have been reported to be soil and irrigation water and, during minimal processing (cleaning and washing), insufficiently disinfected process water (Dobhal, Zhang, Gautam, Fletcher, & Ma, 2015; EFSA, 2014b). The growing cycle for melons varies between 30 to 90 days, which can also expose the produce to variable environmental conditions that can lead to a wide range of unintentional inputs that are potential sources of food safety hazards (EFSA, 2014b). Climatic conditions (e.g. heavy rainfall) that increase the transfer of pathogens from their

reservoirs to the melon and processing plant, have been identified as one of the main risk factors for biological contamination of melons (EFSA, 2014b). The ARC Training Centre for Food Safety in the Fresh Produce Industry will undertake a research project to explore relationships among pathogen characteristics, details of illness outbreaks and climatic conditions (ARCTrainingCentreforFoodSafetyintheFreshProduceIndustry, 2018a). This information may enable the development of predictive models to reduce the number of future foodborne outbreaks, by aiding producers in establishing limits for when fresh produce is cut and harvested. In addition, a deeper understanding of the potential impacts of adverse weather events could enable knowledge-led proactive review of produce sanitising steps. For example, the adjustment of washing parameters for produce grown/harvested during a significant weather event to include additional washing steps and higher concentrations of sanitisers.

The investigation of the recent 2018 *Listeria* outbreak in Australia found that the contamination is likely to have occurred due to adverse weather (localised storm over the farm and subsequent dust storms during the season) increasing the levels of *Listeria* on the fruit prior to harvest (NSWDPI, 2018; NSWFoodAuthority, 2018c). On this occasion and despite following industry best practice, the washing and sanitising procedures in place were not able to remove all the trapped bacteria from the rockmelon surfaces, resulting in a low level of *Listeria* being present (NSWFoodAuthority, 2018c). In the investigation report (NSWDPI, 2018) it is surmised that the sanitising step was effective in reducing *L. monocytogenes* to a very low level. However, as the epidemiological data attests, these low levels of *L. monocytogenes* still resulted in illness when rockmelons were consumed by immunocompromised individuals. The investigation found there was also an opportunity for the introduction of *Listeria* after washing through contact with surfaces or equipment that may have had traces of *L. monocytogenes* (NSWFoodAuthority, 2018c). This includes dust blown from fans used to dry the fruit after washing, and from porous material on packing tables that was not able to be effectively cleaned at the time (NSWFoodAuthority, 2018c).

A recent study by Nyarko et al. (2018) investigated the effect of equipment surface type and cleanliness on the persistence and transfer of *L. monocytogenes* to rockmelons in the packing environment (Nyarko et al., 2018). Not surprisingly, it was found that soiled surfaces supported the survival of higher populations of *L. monocytogenes*, and surface type affected *L. monocytogenes* contamination transferred to melons (Nyarko et al., 2018). Comparison of clean surfaces revealed that foam pads and brushes significantly promoted *L. monocytogenes* persistence compared to conveyor belt materials (polyvinyl chloride, polyurethane, and nitrile rubber) (Nyarko et al., 2018). Foam pads inoculated with *L. monocytogenes* were also found to contaminate significantly more melons than conveyor belts (Nyarko et al., 2018). Similarly, De Abrew Abeysundara et al. (2018) reported that rockmelon flesh and peel extracts at very low concentrations support growth and biofilm formation of *Salmonella enterica* on different food-contact surfaces (De Abrew Abeysundara et al., 2018). Biofilm formation by *Salmonella enterica* was also shown to be affected by surface type and, lowest on buna-n rubber compared to stainless steel, polyethylene and polyurethane surfaces under the majority of conditions tested (De Abrew Abeysundara et al., 2018). The 2018 *Listeria* outbreak investigation report (NSWDPI, 2018) is publicly available and makes several recommendations for industry/growers.

The diversity of production and processing methods in the melon industry make a single, universally applicable approach to food safety planning complicated (Adams et al., 2005). A washing step is generally used to reduce microbial loads on rockmelons. However, surface irregularities such as roughness, crevices and pits increase bacterial adherence and reduce the ability of washing treatments to completely remove bacterial cells (Frank & Koffi, 1990; Ukuku, 2004). Traditionally, chemical treatments have been employed to control pathogens on the rind of rockmelons. Chlorine and its derivatives are the most widely used disinfectants to sanitise rockmelons (Raúl O. Saucedo-Alderete, Joseph D. Eifert, Renee R. Boyer, Robert C. Williams, & Gregory E. Welbaum, 2018), however a number of studies have shown that they have a limited effect on pathogen reduction. For example, it was reported that application of 180 ppm of chlorine, acidified calcium sulfate (ACS: 1.2% Safe2O-ACS50), 1,000 ppm of acidified sodium chlorite (ASC), 80 ppm of peroxyacetic acid (PAA), and a combination of ACS and PAA for 10 min, achieved no more than a 1.5 log

reduction of *Salmonella* from rockmelon surfaces (Fan, Annous, Keskinen, & Mattheis, 2009). In addition, chlorine and its derivatives are affected by organic matter; are corrosive at high concentrations; not stable in diluted solutions and concentrates, and cannot be stored for a long time without losing their antimicrobial activity.

In Australia, the inconsistent use of sanitisers in wash water has been identified as a risk factor in previous melon outbreaks (NSWFoodAuthority, 2014a). Other industry practices are also highly variable, in terms of sanitiser and fungicide use (type/s, concentration and order of application), method of application and monitoring (wet dumping or spray), whether produce is physically brushed and, whether wash water is recirculated (with or without treatment and sanitiser addition). There is also evidence of reduced efficacy of some sanitisers when used in combination with fungicides (FPSC, 2015b). This increases the risk of cross-contamination from wash water and has the potential to impact on food safety (FPSC, 2015b). In Australia, the melon industry is currently undertaking research with the ARC Training Centre for Food Safety in the Fresh Produce Industry into the best practice in food safety on rockmelon farms. Projects include gaining a greater understanding of sanitiser and fungicide use patterns and, assessing whether fungicide and sanitisers that are commonly used to control postharvest contamination interact to mutually augment or suppress microbial activity. Anecdotal evidence suggests that many growers are not aware of existing information on the potential compatibility issues for combining sanitisers and fungicides, which could be addressed through communication and extension action (FPSC, 2015b). In light of the listeriosis outbreak in 2018, Hort Innovation launched an initiative being delivered by the NSW Department of Primary Industries, to monitor and improve industry best-practice (HortInnovation, 2018).

As current postharvest washing and sanitising procedures are unlikely to completely remove bacteria from the rind surface of rockmelons, there has been a concerted international research effort to identify more effective sanitising options for rockmelons and rockmelon contact surfaces. For example, recent studies have been undertaken to investigate the effectiveness of delmopinol (R. O. Saucedo-Alderete, J. D. Eifert, R. R. Boyer, R. C. Williams, & G. E. Welbaum, 2018), levulinic acid and sodium dodecyl sulfate (SDS) (Webb, Erickson, Davey, & Doyle, 2015), cold plasma-activated hydrogen peroxide aerosol (Jiang et al., 2017), peracetic acid (Singh, Hung, & Qi, 2018) and water vapor (steam) at a high temperature (Bezanson, Ells, Fan, Forney, & LeBlanc, 2018; Kwon, Song, & Kang, 2018). Of particular interest is the potential to use water vapor (steam), which would offer major advantages over the use of chemicals, as it utilises a chemically stable gaseous reagent that leaves no residue and is also likely to be economically feasible at a commercial level (Bezanson et al., 2018). Kwon et al. (2018) investigated the effectiveness of superheated steam treatments at 200°C for 30 s on inactivation of *E. coli* O157:H7, *Salmonella* Typhimurium and *L. monocytogenes*-inoculated rockmelons. Under these treatment conditions, populations of the three pathogens on rockmelons were reduced by more than 5 log (Kwon et al., 2018). In addition, Kwon et al. (2018) report that under these treatment conditions there was no deterioration in the colour and texture qualities of the rockmelons. Bezanson et al. (2018) investigated aerated steam sanitisation of rockmelons and reported that exposure for 240 s to aerated steam heated to 85°C achieved a mean reduction in surface-inoculated *L. innocua* of 3.9 ± 0.6 log_{10} CFU/cm^2 and decreased background microorganisms (yeast, moulds, and coliforms) to undetectable levels (Bezanson et al., 2018). In addition, no significant outgrowth of surviving *L. innocua* or yeast and moulds was observed on heat-treated melons during their storage at 4, 7 and 10°C for 14 days (Bezanson et al., 2018). While rind quality was altered under these treatment conditions, edible fleshy portions remained largely unaffected (Bezanson et al., 2018). However, Bezanson et al. (2018) also reported that on treated and untreated rockmelons inoculated with *L. innocua*, *L. innocua* was more proficient at colonizing and growing on aerated steam-treated melons than on those not exposed to heat. This suggests a need for the use of post-sanitisation contamination control hurdles to prevent secondary colonisation.

EFSA published their scientific opinion on the risk factors for melon contamination by *Salmonella*, in the context of the whole food chain, together with available estimates of *Salmonella* occurrence and mitigation options relating to prevention of contamination and the relevance of microbiological criteria (EFSA, 2014b). It was suggested that a Food

Safety Criterion for *Salmonella* on whole melons could be considered as a tool to communicate to producers and processors that *Salmonella* should not be present on the product ([EFSA, 2014b](#)). As the occurrence of *Salmonella* is likely to be low, unless the final product is grossly contaminated, it is highly unlikely that a practical and economical random sample of finished product would result in a positive detection. A positive *Salmonella* test result from whole melons would therefore most likely be indicative of a major breach in GAPs, GHPs, GMPs or HACCP programmes ([EFSA, 2014b](#)). Similarly, a positive *Salmonella* test result could be the result of adverse weather events, such as flooding or a heavy rainfall when water is at a higher risk of contamination. Setting microbiological criteria for *L. monocytogenes* and the return of a positive test result, would likewise provide insight into whether the safety of the product has been compromised by major breaches in food safety management programmes or by other confounding factors. Environmental monitoring for *L. monocytogenes* ([NSWFoodAuthority, 2016a](#)) would also be particularly relevant, as some strains have been found to persist for years or decades in food processing plants; due to their ability to form biofilms, physiological tolerance to sanitation or processing hurdles and/or, survival in niches within the food environment that may be difficult to clean and disinfect (e.g. cracks and crevices of surfaces) ([Buchanan, Gorris, Hayman, Jackson, & Whiting, 2017](#)). Collecting samples for whole genome sequencing (WGS) from food production sites and sharing these data would also aid food processors in understanding the ecology of these pathogens and, would assist identification and traceback during outbreaks. This would be helpful for outbreak investigations for listeriosis, which are particularly difficult because of the sporadic nature of the illness and lengthy incubation period that can complicate and delay identification of the causative food ([Buchanan et al., 2017](#)). In addition, rockmelons typically are not stamped with identifying information on their origin, so cases cannot report which brand they consumed.

In Australia, there is currently limited data on the occurrence of *Salmonella* and *L. monocytogenes* on rockmelons. While there are several published surveys from other countries ([Denis et al., 2016](#); [Luchansky et al., 2017](#); [Zhang et al., 2018](#)), it is difficult to make meaningful comparisons between individual studies as well as to assess the representativeness of these data to estimate the overall levels of contamination likely in Australia. Targeted surveys to determine the occurrence of *Salmonella* and *L. monocytogenes* on rockmelons at specific steps in the food chain would provide useful baseline data. This would enable the establishment of a microbiological benchmark to support food safety decisions within the rockmelon industry. However, it's important to note that microbiological testing alone may convey a false sense of security due to the statistical limitations of sampling plans, particularly in the cases where the hazard presents an unacceptable risk at low concentrations and/or low and variable prevalence ([EFSA, 2014b](#)). As faster, more sensitive and accurate detection technologies and tools become available, environmental and end-product testing will become a more powerful tool for the rockmelon Industry.

Currently, the risk of contamination of rockmelons cannot be reduced sufficiently to make consumption safe for highly vulnerable populations. As a precaution, FSANZ ([FSANZ, 2012a](#)), the NSW Food Authority ([NSWFoodAuthority, 2018d](#)) and the NSW Department of Health ([NSWDoH, 2018](#)) recommend that vulnerable people should avoid consuming rockmelon. Amongst the risk minimisation strategies recommended by NSW DPI in their *Listeria* outbreak investigation report ([NSWDPI, 2018](#)), was the need for a unified communication strategy across regulatory bodies and industry associations to further educate vulnerable consumers and their carers about the risks of listeriosis and practical solutions to maintaining a balanced diet. This communication strategy would include recommending that vulnerable populations do not consume rockmelons ([NSWDPI, 2018](#)), consistent with current consumer advice ([FSANZ, 2012a](#); [NSWDoH, 2018](#); [NSWFoodAuthority, 2018d](#)).

8.2 Berries

Fresh, frozen and freeze-dried berries are vulnerable to processing (i.e. cleaning or washing) and therefore are most often consumed whole and unprocessed, exposing consumers to a risk of food poisoning. In addition, cooling and freezing processes are not considered suitable for the control of viruses as they do not reduce virus infectivity to levels

considered safe. For example, on berries, hepatitis A is resistant to the freeze-drying process ([Butot, Putallaz, Amoroso, & Sánchez, 2009](#)) and there is negligible effect on infectivity for up to 90 days at -20°C during frozen storage ([Butot, Putallaz, & Sánchez, 2008](#)). Consequently, it is critical to ensure that these products are not contaminated with foodborne viruses, or bacterial pathogens such as *Salmonella* and STEC.

Hepatitis A and norovirus have both been associated with many foodborne outbreaks linked to fresh and frozen berries worldwide ([Palumbo, Harris, & Danyluk, 2016](#); [Tavoschi et al., 2015](#)). There have been several outbreaks of hepatitis A in Australia that have been caused by imported frozen berries (Appendix 3). Unlike hepatitis B and C, hepatitis A infection does not cause chronic liver disease and is rarely fatal, but it can cause debilitating symptoms and acute liver failure, which is often fatal ([WHO, 2017](#)). Humans are considered the only source of hepatitis A, which is transmitted via the faecal-oral route by either person-to-person contact or consumption of contaminated food or water. Asymptomatic and symptomatic infected persons are generally unaware they present a hazard at the time most virus is shed in faeces ([FSANZ, 2015](#)). Hepatitis A is a particular risk in RTE food products sourced from countries in which the virus is endemic. Hepatitis A is not endemic in Australia and therefore infection resulting from domestically contaminated food or water or an infected food handler is rare.

In 2015, FSANZ published a risk statement on hepatitis A virus and imported RTE berries. The risk statement concluded that, hepatitis A virus in RTE berries produced and handled under GAPs and GHPs is not a medium to high risk to public health ([FSANZ, 2015](#)). The risk statement ([FSANZ, 2015](#)) was provided to the Department of Agriculture and Water resources (DAWR), which is the enforcement agency for imported food. DAWR administers two sets of requirements for imported food. Food imported into Australia must meet biosecurity requirements under the Biosecurity Act 2015 and is subject to the Imported Food Control Act 1992 to meet requirements for food safety and compliance with Australia's food standards. Recently, the Imported Food Control Amendment Bill 2018 (["Imported Food Control Amendment Bill 2018," 2018](#)) was passed and introduces amendments to the Imported Food Control Act 1992. Amongst these amendments are that Australia and the exporting country have equivalent food safety systems; and Australia and the exporting country conduct equivalent monitoring of the food they regulate. DAWR has powers under the Imported Food Control Act 1992 to operate a food safety inspection program known as the Imported Food Inspection Scheme (IFIS). Currently, imported berries that are not intended to undergo further processing are tested for *E. coli* ([DAWR, 2018](#)). However, the use of *E. coli* as a faecal indicator has limited value due to its widespread prevalence in the environment and variable correlation with human enteric viruses ([Li, Butot, Zuber, & Uyttendaele, 2018](#)).

There is no routine or regular monitoring of berry fruits for the presence of hepatitis A and norovirus globally. There is also very limited prevalence data on the rates of contamination of berries by these viruses in the peer-reviewed literature. This is largely due to the constraints of viral ribonucleic acid (RNA) extraction and the low levels of virus in contaminated food and water. Quantitative real-time polymerase chain reaction (qRT-PCR) has been one of the most promising detection methods for hepatitis A virus and norovirus quantification in soft berries (Fraisse et al., 2017). The International Organisation for Standardisation technical specifications (ISO/TS), ISO/TS 15216-1 and 15216-2 use real-time polymerase chain reaction (RT-PCR) technology for both quantitative determination and qualitative detection of hepatitis A virus and norovirus in foodstuffs (including soft fruits) ([ISO, 2013](#), [2017](#)). However, this approach suffers from some serious drawbacks in detecting enteric viruses in berries ([Li et al., 2018](#)) and a number of recent publications have aimed to improve the robustness and sensitivity of these analytical procedures ([Hida, Papafragkou, & Kulka, 2018](#); [Summa & Maunula, 2018](#)).

Due to the inherent difficulties in detection of viruses in berries, it is currently not possible to rely on sampling and end-product analysis as effective control measures. Therefore, effort must be focused on preventive pre-harvest and post-harvest measures ([Rajkovic et al., 2017](#)). Contamination may occur at several points along the farm-to-fork continuum

through human faecal contaminated irrigation water, manual handling during harvest, postharvest processing, or food preparation by infected (symptomatic or asymptomatic) food handlers practicing poor personal hygiene.

EFSA recently prepared a scientific opinion on the risks posed by norovirus in berries (EFSA, 2014a). Norovirus infections are highly contagious and are a leading cause of gastroenteritis in Australia and worldwide. Noroviruses are highly infectious and are spread from the vomit or faeces. The EFSA Panel was tasked with identifying the main risk factors for norovirus in berries, recommend possible specific mitigating options and recommend, if considered relevant, microbiological criteria for norovirus in berries (EFSA, 2014a). EFSA reported that the risk factors for norovirus during primary production and minimal processing though poorly documented are likely to include environmental factors (e.g. heavy rainfall), use of sewage-contaminated agricultural water and contamination and cross-contamination by harvesters, food handlers and equipment at harvest or post-harvest. In terms of specific mitigating options to reduce the risk for humans posed by norovirus in berries, it was recommended that appropriate implementation of food safety management systems should be the primary objective of producers from farm-to-fork and include GAPs, GHPs and GMPs. EFSA concluded that there was insufficient evidence to justify the establishment of microbiological criteria for norovirus for fresh or frozen berries. It was recommended that targeted surveys be conducted on the occurrence of norovirus in different types of berries both at primary production, after minimal processing (including freezing) and at the point of sale. Where possible, it was recommended that these surveys should use methods which provide an indication of virus infectivity, together with studies to identify the level of hazard control and efficacy of application of food safety management, including HACCP, that has been achieved at different stages of production systems. EFSA concluded that the collection of appropriate data and subsequent risk-based development of microbiological criteria to support improved control of norovirus in frozen raspberries and strawberries should be considered as a priority.

In Australia, a survey was undertaken to establish baseline information about current food safety management practices during growing, harvesting and processing of strawberries (DEDJTR/FSANZ, 2016). The survey involved discussion and observation of 33 Victorian strawberry growers during the 2014/15 growing season (DEDJTR/FSANZ, 2016). Strawberries were sampled in parallel with the survey to test for the presence of the faecal indicator bacterium *E. coli*. Of the 33 farms, 44% (14/32) did not have a quality assurance (QA) system in place, 56% (18/32) had one or more QA systems in place and, no data about QA systems was recorded for one farm. While overall the survey findings indicated that food hygiene was generally well managed by Victorian strawberry growers, the survey did highlight some opportunities for guidance, improvement and/or further investigation:

- The dissemination of basic food safety awareness guidance material is likely to assist growers understand and manage any potential food safety risks, including the risk of human-virus contamination.

- Investigate whether the occasional presence on crops of feral animals such as ducks, birds, rabbits, foxes and mice; poses a food safety concern.

- Only use potable water for overhead spray irrigation.

- Seek an alternative method to the use of cloth towels for drying strawberries.

- Instructions regarding glove use should clearly inform staff of the reasons for this requirement, together with advice on suitable complementary actions to ensure hygienic fruit handling. The provision of hand sanitisers for use by food handling staff is beneficial against bacteria. However, they are relatively ineffective against human viruses such as norovirus. The use of clean gloves or handwashing is preferred.

- Investigating the need for toilets to be available in the field for workers.

- Implement food identification labelling for second-grade strawberries.

- Communicate the advantages of food safety systems. It is recommended to provide this information for the consideration of the growers that currently do not have a food safety system in place.

In addition, the survey indicated that there may be a perception amongst farmers that human viruses are not a potential food safety risk for the production of horticultural produce in Australia, and are only a risk for overseas countries farmers to manage (DEDJTR/FSANZ, 2016).

8.3 Fermented nut cheeses

Fermented nut cheeses have emerged as a plant-based alternative to dairy cheese for those seeking a vegan or vegetarian diet. Fermented nut cheeses are made from cashews, macadamias, almonds or other nuts by soaking and grinding with water, followed by fermentation (Tabanelli et al., 2018). Other ingredients, such as spices, herbs, lemon juice, and salt etc, can be added after fermentation, depending on the recipe (Tabanelli et al., 2018). Fermented nut cheeses are a high risk potentially hazardous food, as evidenced from previous illness outbreaks associated with these products. In 2013, consumption of raw cashew cheese was the source of an outbreak of *Salmonella* Stanley infections across three states in the U.S.A (CDC, 2014b). This outbreak resulted in 17 cases and led to three hospitalisations.

In NSW, fermented nut cheese production is not regulated and there is little knowledge of the processing steps and quality and hygienic standards adopted by producers. There is also very little in the published literature on this topic. Recently, the BC Centre for Disease Control (BCCDC) of British Columbia reviewed food safety processes and offered guidance on the manufacture of fermented cashew nut cheese (BCCDC, 2017). The BCCDC (2017) reported that while there are many variations in the actual production of fermented nut cheeses, the overall process involves:

1. Soaking of nuts to soften
2. Mechanical grinding / blending of nuts (before or after fermentation step)
3. Addition of lactic acid bacteria culture and other ingredients for flavour
4. Fermentation
5. Drying and molding (shaping) of cheese
6. Packaging and storage.

The BCCDC (2017) concluded that from a food safety perspective, all of the steps in the process carry inherent risk, either from the source ingredient or process method (BCCDC, 2017). Risks associated with tree nuts and ground nuts (peanuts) are well established. Nuts and seeds are both known sources for *Salmonella*, the bacterial hazard of primary concern in this product (BCCDC, 2017). The BCCDC (2017) report recommends that ingredients should be sourced from suppliers that can provide assurance that ingredients have been screened and tested free of hazards of concern. However, testing does not detect all low or intermittent levels of bacteria of concern on nut products, therefore cooked, rather than raw, nuts are recommended (BCCDC, 2017). The BCCDC (2017) offers specific advice around methods to reduce bacterial levels, including frying, wet and dry roasting and blanching. The BCCDC (2017) recommends that a hot water blanching step be used prior to soaking of cashews and other types of tree nuts, consisting of pouring boiling water over the nuts and ensuring that the temperature does not drop below 90°C for at least two minutes. As an example, the BCCDC (2017) cites the first process step listed in one commercial production of fermented nut cheese; which is a 10-minute soak of nuts in 90°C water.

A summary of the recommendations on fermented nut cheese production from the BCCDC (2017) is listed below:

1. Ingredients must be sourced from suppliers that can verify products are testing free of *Salmonella*, *Listeria*, *E. coli* and other hazards;
2. Sanitary facilities and handling are required for manufacture of this product. If the cashews or other nuts are mechanically chopped or blended there must be a sanitation plan in place to limit contamination from this processing and handling step;

3. Rejuvulac water (wild fermentation cultures that are created through soaking of wheat berries or other seeds) is not recommended for the manufacture of nut cheeses. Biological hazards (e.g. *Salmonella*) are difficult to control in sprouted seeds and significant risk of illness exists associated with sprouted seeds;

4. Back-slopping (using a previous culture), use of kombucha, yogurt, miso paste, probiotic pills, or pickling brine as a starter culture is not recommended;

5. Nuts should be heat pasteurised (pasteurised is preferable over raw) prior to the soaking / fermentation step;

6. After heat pasteurizing, nuts should be cooled from 60°C to 20°C in two hours, and further cooled from 20°C to 4°C in four hours;

7. Nuts soaked in water must have an additional control step to minimise growth of *Salmonella* by either (1) refrigeration, or if soaking nuts at room temperature (2) acidification of the water to below pH 4.6, or (3) addition of lactic acid bacteria (LAB) starter culture with fermentation verification at 24 hr;

8. Fermentation verification: pH tests of the initial cashew ferment (0 hrs), and ferment process (at 24 and 48 hrs) must be provided, and sufficient to show an active fermentation with 2 days is established with pH dropping to below 4.4 by end of 2nd day. Use of a pH meter is recommended;

9. The final pH of the fermented nut cheese should be assessed to determine shelf-life and storage conditions under reduced oxygen packaging conditions. Cheeses with acidity greater than a pH of 4.4 or water activity greater than 0.94 should be held refrigerated to control for the risk of *L. monocytogenes*.

Further to these recommendations, in Victoria, the Department of Health and Human Services has produced a supplement for businesses manufacturing high-risk foods using various acidification methods (DHHS, 2017). The supplement recommends that producers of acidified foods should aim for a pH of 4.2, in order to ensure that the products remain well under a pH of 4.6 throughout their entire shelf life. This accounts for the fact the pH of the product may rise, if the acid is neutralised by the food or if it is absorbed into the food. As some food-poisoning bacteria; such as *Salmonella* spp., *E. coli* and *L. monocytogenes*, can still grow in food below pH 4.6, a combination of other control measures are advised. Specifically, the supplement states that food safety in acidification and fermentation requires:

- good quality, undamaged, raw materials

- contamination prevention (before, during and after processing)

- well-controlled acidification and fermentation.

It should be noted that outbreaks have also been associated with nut spreads (CDC, 2014a, 2016) and some of the guidance offered in this section may also be applicable to these products.

8.4 Plant-based meat alternatives

Plant-based meat alternatives represent a potential alternative sustainable source of valuable dietary proteins for vegans, vegetarians and those consumers seeking to reduce their meat consumption. Frequently cited reasons for consumption of these products are health and nutritional benefits, environmental considerations, religious beliefs and animal welfare (Haverstock & Forgays, 2012). Data from market researcher Euromonitor International has shown Australia's packaged vegan food market is set to reach $215 million by 2020, making the Australian market the third-fastest growing vegan market in the world after the United Arab Emirates and China (SBS, 2018).

A number of alternative protein sources are now being explored for the production of food (Alexander et al., 2017) and numerous plant-based products are currently available on the market domestically and internationally (Mattila et al., 2018). All foods containing proteins have the potential to be allergenic. Characterisation of potential protein-based allergenic hazards in novel food ingredients is essential to support effective risk assessment (Pali-Schöll et al., 2018). Accurate product labelling is also essential to ensure that people affected by food allergies can identify potential

allergens. In Australia, all food retailers, manufacturers and importers are responsible for managing the presence of allergens in food. Food businesses must meet the labelling requirements set out in Standard 1.2.3 (Mandatory Warning and Advisory Statements and Declarations) of the Food Standards Code.

One meat alternative currently available is mycoprotein, which is currently only sold under the trade name Quorn™. To produce mycoprotein, the filamentous fungus *Fusarium venenatum* is grown in a continuous fermentation process and the resulting biomass is heated, concentrated and extracted to generate a mycelial paste (mycoprotein) (Wiebe, 2004). The mycoprotein can be further combined with a binding agent such as egg albumin and various spices or flavourings, depending on the desired final product (Wiebe, 2004). Quorn™ produce over 100 different products and mycoprotein is used as an ingredient in every product (Quorn, 2018). Quorn™ products were launched in 1985 in the UK and have since secured considerable consumer acceptance, with products now also available in 17 other countries (Quorn, 2018). There are currently 16 Quorn products available in Australia (Quorn, 2018). Some consumers have reported adverse reactions after eating mycoprotein-based products. The Center for Science in the Public Interest (CSPI) analysed self-reports of adverse reactions to mycoprotein via a Web-based questionnaire, resulting in the collection of 1,752 adverse reaction reports of allergic and gastrointestinal symptoms, with some people experiencing both (Jacobson & DePorter, 2018). Mycoprotein is not classified as an allergen in any of the countries in which it is currently sold. Research in Europe suggests that while most consumers can eat these products safely, about 1 in 100,000 to 200,000 people may react to them (FSANZ, 2011). In Australia, a 17-year old girl experienced severe hypotensive anaphylaxis immediately after eating vegetarian fajitas containing mycoprotein (Dzeladini, Chan, & Kummerow, 2017). Subsequently, the patient had a skin prick test and was shown to be strongly positive to mycoprotein (Dzeladini et al., 2017). The patient had a history of previous allergic reactions to some mushrooms (mushrooms are the fruiting bodies of certain types of fungi) and displayed a positive skin prick test to *Alternaria* mould and pollens (Dzeladini et al., 2017). Reports suggest that patients sensitised to environmental mould may be at risk of a severe allergic reaction, even after their first ingestion of mycoprotein (Dzeladini et al., 2017). The consumption of products derived from fungal fermentation continues to increase and patients with significant sensitisation to mould should be made aware of the risk of an allergic reaction. In Australia, either mycoprotein or Quorn™ will be listed in the ingredients list on food labels and consumers can check the label and avoid products that may be of concern to them (FSANZ, 2011). However, few people may realise that mycoproteins are derived from fungi. Consideration should be given to labelling these foods with 'caution in individuals with mould allergy'. In the U.S.A, Quorn products must be labelled with the statement: "Mycoprotein is a mould (member of the fungi family)." However, Quorn products may also be served at restaurants, cafes, and other foodservice businesses and there may be no information provided to consumers to inform them that they are eating mycoprotein derived from fungi.

Allergic reactions to the legume family are common and comprehensive reviews have identified the major allergens and cross-reactivity within this family (Verma, Kumar, Das, & Dwivedi, 2013). The major legumes include peas, beans, lupin, lentils, peanut and soy. A number of legume-based meat alternative products have been launched domestically and internationally. In Australia, Funky Fields has produced a soy and wheat gluten-based mince product called "Minced", which became available in the meat section of various supermarkets in 2018. Beyond Meat was founded in 2009 and produces a range of pea and soy-based meat alternatives that are available for purchase in packaged form as well as in retail-prepared dishes, in the U.S.A and Hong Kong. Beyond Meat have recently partnered with a group of distributors to enter over 50 other countries, including Australia (BeyondMeat, 2018).

Impossible™ Burger was established in 2011 and is currently available in restaurants in the U.S.A and Hong Kong. Earlier this year, Air New Zealand announced it will be serving the Impossible Burger to business class passengers on flights from Los Angeles to Auckland (AirNewZealand, 2018). The key ingredient that gives the Impossible Burger its meaty flavour and aroma is soy leghaemoglobin from the root nodules of soybean plants, which is produced on an industrial scale in genetically engineered yeast (*Pichia pastoris*). The primary concerns for food safety from any

recombinantly expressed protein are whether the protein itself is allergenic, whether the protein is cross-reactive due to similarity to another protein, whether the protein is a toxin, or whether insertion of the gene alters the quantity of endogenous allergens if the host is commonly allergenic (Y. Jin et al., 2018). Jin et al. (2018) evaluated the soy leghaemoglobin and *Pichia pastoris* proteins for their potential risk of allergenicity and toxicity in accordance with the CODEX Alimenterius Commission 2003/2009 guidelines for genetically modified foods and for novel food ingredients (Y. Jin et al., 2018). The potential toxicity assessment recommended by CODEX includes a review of toxins or toxicity of the source organism and a comparison of the protein amino acid sequence identity between the novel protein and known protein toxins. Tests to measure the stability of the protein in an *in vitro* gastric digestion model are also used to judge potential risks of potential allergenicity or toxicity of the protein. From their study, Jin et al. (2018) concluded that foods containing recombinant soy leghaemoglobin produced in *Pichia pastoris* are unlikely to present an unacceptable risk of allergenicity or toxicity to consumers (Y. Jin et al., 2018). Subsequently, Fraser et al. (2018) evaluated the potential genotoxicity of soy leghaemoglobin protein expressed in *Pichia pastoris* by conducting a bacterial reverse mutation test (Ames test) and an *in vitro* chromosome aberration test. Systemic toxicity was assessed in an *in vivo* 28-day dietary study in male and female rats (Fraser, Shitut, Agrawal, Mendes, & Klapholz, 2018). Fraser et al. (2018) concluded that collectively, the *in vitro* and *in vivo* results suggest that soy leghaemoglobin and *Pichia pastoris* proteins from the production host, raise no issues of toxicological concern under the conditions tested.

As new plant-based meat alternative products are developed, potential protein-based allergenic hazards will need to be characterised to support effective risk assessment and product labelling.

8.5 Microgreens

Microgreens are edible plants of any species, grown beyond the point of harvest of sprouted seeds, and normally include the cotyledons and first true leaves. They are marketed for their attractive appearance and intense flavour and normally consumed raw in relatively low volumes, as garnishes or mixed into salads and sandwiches. Unlike sprouts, foodborne outbreaks have not been associated so far with the consumption of microgreens. Currently microgreen production is not protected from microbial contaminants by the same Australia New Zealand Food Standard (Standard 4.2.6) regulating the production and processing of seed sprouts. An important distinction between sprouts and microgreens is the production process, with microgreens being typically grown in soil or soil substitutes (such as peat moss, perlite, vermiculite, rock wool, or other fibrous materials) and harvested above the soil or substrate line. However, the routes of pre-harvest contamination for microgreens are likely to be the same as for sprouted seeds, i.e. from the seed or from irrigation water, suggesting that similar critical control points and barriers for microgreens should be in place as for sprouted seeds (Wright & Holden, 2018).

Xiao et al. (2015) investigated the proliferation of *E. coli* O157:H7 in radish microgreens (7 days after seeding) produced from inoculated seeds grown in peat moss–based soil-substitute and hydroponic production systems (Xiao et al., 2015). *E. coli* O157:H7 was shown to survive and proliferate significantly during microgreen growth in both production systems, with a higher level in the hydroponic production system. At the initial seed inoculation level of 3.7 log CFU/g, *E. coli* O157:H7 populations on the edible part of microgreen plants reached 2.3 and 2.1 log CFU/g (overhead irrigation and bottom irrigation, respectively) for microgreens from the soil-substitute production system and reached 5.7 log CFU/g for those hydroponically grown. Examination of the spatial distribution of bacterial cells on different parts of microgreen plants showed that contaminated seeds led to systematic contamination of whole plants. These results indicate that seeds could be an important source of contamination in microgreen production. Similarly, Reed et al. (2018) reported that artificially contaminated seeds and irrigation water led to the systematic contamination of microgreen plants by *Salmonella* (Reed et al., 2018).

It has been suggested that seeds used in microgreen production should receive precautionary sanitary treatments for eliminating pathogenic bacteria such as those recommended for sprout production (Kyriacou et al., 2016). In addition,

aside from seeds, growing media could also represent a potential source of microbial contamination for microgreens (Di Gioia, De Bellis, Mininni, Santamaria, & Serio, 2017).

9. Conclusion

Globally, a significant increase in foodborne disease outbreaks or cases associated with consumption of fresh produce has been reported. While Australia has not experienced the same magnitude and severity of outbreaks described in other developed countries, the risk potential exists.

In the majority of foodborne illness cases and outbreaks linked to fresh produce, the contamination is brought into the processing environment and subsequently disseminated over different batches (Murray, Wu, Shi, Jun Xue, & Warriner, 2017). The Australian fresh produce sector is not regulated by means of national primary production and processing standards. The scope of the NSW Plant Products Food Safety Scheme also does not include whole raw fruits and vegetables, which are not intended to be further processed. Despite the implementation of voluntary quality assurance programs implemented by the industry for on-farm activities, foodborne outbreaks linked to contaminated fresh or minimally processed fruits and vegetables continue to be reported in Australia. In the U.S.A, the FDA has moved away from oversight of produce safety through voluntary guidance (e.g. GAPs) and other industry-driven initiatives, to establishing science-based minimum standards for the safe production of produce; including standards for growing and harvesting. The FDA estimates that about 332,000 total illnesses per year will be prevented by the provisions of this new Rule (FDA, 2016). In Australia, regulation at the primary production stage may be warranted for high-risk horticultural sectors in which an increased level of oversight could improve current food safety management systems. Within NSW, a project is currently being undertaken by DPI (DPI, 2018) to review high-risk horticulture primary production within the rockmelon, lettuce and berry industries. The aim of this project is to establish baseline requirements with industry for businesses engaged in producing and packing high risk horticultural produce (DPI, 2018). Similarly, FSANZ has commenced work into regulatory and non-regulatory measures for five high risk horticulture sectors at the request of Food Ministers: ready to eat, minimally processed fruits and vegetables, fresh leafy green vegetables, melons, berries and sprouts (theForum, 2018).

It is important to emphasise that while on-farm GAPs contribute to preventing pathogens entering the fresh produce chain, they cannot be relied upon completely due to the open nature of farming. This places a strong requirement on producers to implement effective post-harvest decontamination interventions. As discussed within this risk assessment, current post-harvest washing and sanitizing procedures have a number of limitations and various novel decontamination methods have been evaluated for their effectiveness and potential adoption by the produce industry (e.g. steam treatment of melons). The plant products industry will be further supported in food safety management decisions by access; where lacking, to baseline microbiological survey data on hazard prevalence and levels along the plant product supply chain in Australia. In addition, as surveillance systems (WGS and bioinformatics) continue to improve, food producers and processors will increasingly be capable of expanding their efforts in environmental and finished product testing. The information generated would aid industry in the identification of system weaknesses. In addition, early adoption of these surveillance systems would better prepare Industry if and/or when sequencing and testing standards are imposed upon domestic or international supply chains.

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