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Food and Agriculture
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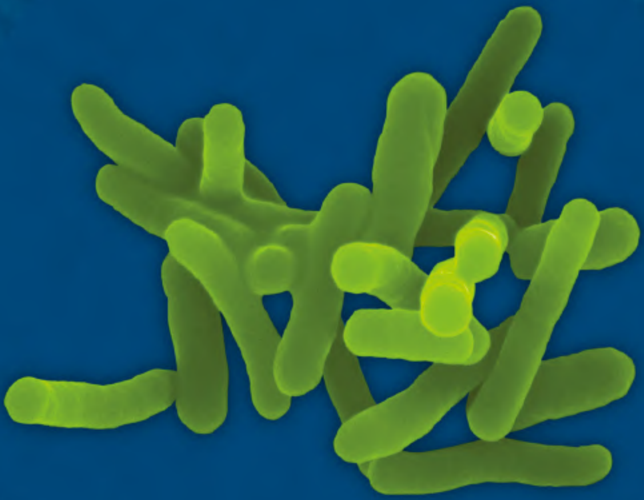
Prevention and control of microbiological hazards in fresh fruits and vegetables
Part 4: Specific commodities

Meeting report



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MICROBIOLOGICAL RISK
ASSESSMENT SERIES

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Prevention and control of microbiological hazards in fresh fruits and vegetables
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Food and Agriculture Organization of the United Nations
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Abbreviations and acronyms

CAC	Codex Alimentarius Commission
CCFH	Codex Committee on Food Hygiene
CEA	controlled environment agriculture
CFU	colony forming units
COD	chemical oxygen demand
FAO	Food and Agriculture Organization of the United Nations
GAPs	good agricultural practices
GHPs	good hygiene practices
GMPs	good manufacturing practices
HICs	high-income countries
HACCP	hazard analysis and critical control points
JEMRA	Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment
LMICs	lower-middle-income countries
MAP	modified atmosphere packaging
NACs	numerous natural antimicrobial compounds
PAA	peracetic acid
PFU	plaque forming units
STEC	Shiga toxin-producing *Escherichia coli*
UV	ultraviolet
UV-C	ultraviolet C
WHO	World Health Organization

Declaration of interests

All participants completed a Declaration of Interests form in advance of the meeting. The interests declared were not considered by FAO and WHO to present any conflict in light of the objectives of the meeting.

All the declarations, together with any updates, were made known and available to all the participants at the beginning of the meeting. All the experts participated in their individual capacities and not as representatives of their countries, governments or organizations.

Executive summary

BACKGROUND AND OBJECTIVE

In 2019, following a request from the Codex Committee on Food Hygiene (CCFH), the Codex Alimentarius Commission (CAC) approved new work at its 42nd session on the development of guidelines for the control of Shiga toxin-producing *Escherichia coli* (STEC) in leafy vegetables and in sprouts.[1]

To support the work of CCFH and to update and expand the information available in MRA14,[2] JEMRA convened a series of expert meetings on preventing and controlling microbiological hazards in fresh fruits and vegetables.

In September 2021, the JEMRA meeting on the Prevention and Control of Microbiological Hazards in Fresh Fruits and Vegetables reviewed relevant measures for control of microbiological hazards from primary production to point of sale in fresh, ready-to-eat, and minimally processed fruits and vegetables, including leafy vegetables, and identified problem areas and subsequent measures to address and avoid potential microbiological contamination.[3]

In November 2021, a subsequent meeting was held with a subset of the JEMRA expert committee to collect, review and discuss relevant measures for the control of microbiological hazards in sprouts, from the production of seeds for sprouting to the production of sprouts and point of sale.[4]

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[1] FAO & WHO. 2018. *Codex Alimentarius. Report of the fiftieth session of the Codex Committee on Food Hygiene.* Rome. www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-50%252FReport%252FREP19_FHe.pdf

[2] FAO & WHO. 2008. *Microbiological hazards in fresh leafy vegetables and herbs.* Microbiological Risk Assessment Series No. 14. Rome. www.fao.org/publications/card/en/c/819bd604-e5f9-5ee5-8bd4-3a9b14d39bed/

[3] FAO & WHO. 2021a. *Summary report of the Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment on the Prevention and Control of Microbiological Hazards in Fresh Fruits and Vegetables (Part 1: Administrative procedures, meeting scope/objectives, data collection; Part 2 General principle and fresh fruits and vegetables).* Rome. www.fao.org/3/cb7664en/cb7664en.pdf

[4] FAO & WHO. 2022. *Summary report of the Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment on the Prevention and Control of Microbiological Hazards in Fresh Fruits and Vegetables (Part 3: Sprouts).* Rome. www.fao.org/3/cb8201en/cb8201en.pdf

The purpose of this final meeting was to reconvene the expert committee to collect, review and discuss relevant commodity-specific interventions in all other fresh fruits and vegetables from primary production to point of sale.

SCOPE

The objective of the meetings was to evaluate commodity-specific interventions used at all stages of fresh fruit and vegetable production from primary production to post-harvest activities, transportation, point of sale, and consumer use. Emphasis was placed on the identification and evaluation of interventions used throughout the world to reduce microbiological hazards of fresh fruits and vegetables that contribute to the risk of foodborne illnesses, taking into consideration their effectiveness, practicality and suitability.

The expert committee addressed four subdivided commodity groups: 1) leafy vegetables and herbs, 2) berries and tropical fruits, 3) melons and tree fruits, and 4) seeded and root vegetables. These commodities were grouped based on similarity in physical characteristics, intervention measures, and the potential volume of published literature to be reviewed. Interventions were identified for specific commodities against various target pathogens (including bacteria, parasites and viruses) and indicator organisms in the following categories:

- Intervention stage (primary production [open field or protected facilities], post-harvest handling, minimal processing, distribution, retail, and consumer handling)
- Intervention type (physical, chemical or biological)
    - Physical interventions included: ultrasound, UV, high-pressure processing, irradiation, pulsed light, plasma and others.
    - Chemical interventions included: natural antimicrobials, chlorine-based chemicals, chlorine alternatives (e.g. organic acids, peracetic acid), modified atmosphere packaging (MAP), gas treatments (e.g. ozone, chlorine dioxide) and others.
    - Biological interventions or biocontrols included: bacteriophages, protective cultures and others.

The scientific literature assessed included studies published between 2008 and early 2022 that were aimed at interventions meant to reduce levels of microbial contamination of fresh fruits and vegetables. The available studies describing physical, biological, chemical, and multiple hurdle technologies were identified using scoping review methodology. Relevant search terms, identified by the JEMRA expert committee, were used to develop a search algorithm consisting of fresh produce (68 terms), pathogen (49 terms), and intervention (143 terms) constructs. A full search in PubMed and limited searches of Web of Science and CABI abstracts databases was conducted. The identified records were deduplicated and imported into Covidence software for further screening and data extraction. After a review of 3 931 references at the title and abstract level, and 1 097 references at the full-text level, a database of 488 relevant studies resulted. The database included studies on the effectiveness of physical and biological interventions in the fresh produce value chain and contained information about intervention, studied pathogens, commodity groups, and types of produce. Given the large number of studies on chemical interventions in the scientific literature, recently published comprehensive reviews were used in the assessments.

For each intervention, the experts reviewed available published literature and data, and assessed if the intervention showed efficacy for different commodities. If an intervention showed efficacy, experts identified how much efficacy was shown and considered factors such as consistency in levels of reduction, prevention of growth if the pathogen is likely to grow on the target commodity, likelihood of being used alone or in combination with other interventions, and practical merit. Several criteria were considered in the assessment of the interventions, including the scale at which research was performed (e.g. laboratory, pilot plant, commercial scale), rigour of both experimental design and data analysis, and practical merit of the proposed approach or technology.

Factors considered in the assessment of practical merit included potential cost, availability of resources, environmental impact, difficulties in performing the task, training needs, regulatory hurdles, consumer acceptance, and recognition that each of the factors are likely to vary across geographies. Applicability to similar commodities or pathogens, when no data was available, was also assessed.

CONCLUSIONS WERE DERIVED FROM AN ASSESSMENT OF PUBLISHED RESEARCH ON COMMODITY-SPECIFIC INTERVENTIONS USED AT ALL STAGES OF FRESH FRUIT AND VEGETABLE PRODUCTION.

All fruits and vegetables

- The application of preventive measures such as good agricultural practices (GAPs) and good hygiene practices (GHPs) during primary production remains the most effective means of reducing the risk of contamination with human pathogens in all fruit and vegetable commodities. Post-harvest activities require GHPs, good manufacturing practices (GMPs), and a hazard analysis and critical control point (HACCP)-based system to prevent microbiological contamination, reduce cross-contamination, or avoid pathogen growth during different post-harvest handling steps. These preventive measures include effective training, personal hygiene of those that handle fresh produce, and sanitary facilities and food safety resources that must be provided so all workers can reduce risks.[5]

- Irrigation water of poor or variable microbiological quality is a major risk factor during fruit and vegetable production. Treatment may be advisable to ensure the consistent removal of microbiological hazards if there is a need for this water to contact the harvestable part of the crop and there are known risks. Where a sufficient supply of water treated by conventional methods (e.g. nutrient removal, chlorination) is unavailable, alternative means to ensure consistent water quality may be needed. For example, treatment using UV or filtration-based systems can reduce populations of bacterial pathogens by up to 6 log in irrigation water, thereby reducing the risk of contaminating the growing plants. While such treatments are effective, practical considerations have hampered their application, notably access to electricity in field settings, controlling flow rates, or the cost of the technologies.
- The microbiological quality of process water is of critical importance due to the risk associated with potential cross-contamination during post-harvest handling and processing operations. Extensive research on biocides to inactivate microorganisms at each step has been conducted. Where there is a reliance on biocides, validation of treatments for process water under pilot or commercial conditions is desirable but rare.
- Several physical methods (e.g. UV, plasma, pulsed light, ultrasound) have been evaluated alone or in combination with other processes or antimicrobial compounds to assess their potential for the disinfection of process water. Some of this research has led to the identification of promising treatments that not only inactivate spoilage microorganisms and/or human pathogens in process water but also on the surface of produce. However, most of this work has remained experimental, and there is scant evidence of industry uptake.

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[5] FAO & WHO. 2021a. *Summary report of the Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment on the Prevention and Control of Microbiological Hazards in Fresh Fruits and Vegetables (Part 1: Administrative procedures, meeting scope/objectives, data collection; Part 2 General principle and fresh fruits and vegetables).* Rome. www.fao.org/3/cb7664en/cb7664en.pdf

- Lack of industry uptake of interventions indicates a need for future research to more carefully address the practicality of new technologies, and to examine their performance under conditions that either closely simulate in-field production, on-farm packing and commercial processing, or by experimentation in commercial-processing facilities. Research should include more thorough examination of treatment effects on shelf-life and sensory quality, which are often overlooked in laboratory-based research.
- Of note, public health data indicates that outbreaks of illness caused by viral or parasitic pathogens are common, but that research on interventions disproportionately addresses control of bacterial pathogens. Survival of viral and parasitic pathogens is typically very different from bacterial pathogens. Additional research focused on non-bacterial pathogens that are implicated in outbreaks would be valuable for assessing the effectiveness of interventions.

Leafy vegetables and herbs

- None of the post-harvest interventions intended for the treatment of whole produce immediately after harvest (e.g. washes, post-processing chemical and physical treatments) reliably deliver significant reductions in human pathogens.
- Irradiation (i.e. gamma, electron beam, X-ray) is the most effective post-harvest treatment against human pathogens on fresh and fresh-cut leafy vegetables. Technology is likely to have similar results in other commodities, but doses will need to be developed for commodity and target pathogens (bacteria, virus, parasite). Reductions in excess of 5 log or complete eradication have been reported with a range of commodities. There are few technological limitations to the use of irradiation, but the cost and adverse consumer response continue to hinder commercial applications.
- Experimental evidence suggests that electrolyzed water in combination with other physical treatments, including ultrasound or exposure to UV, can reduce bacterial pathogens by 3 to 5 log on the surface of leafy vegetables. Potential impediments to commercial applications include engineering complexity and cost. Moreover, little data is available regarding efficacy against viruses or parasites.
- Application of bacteriophage or phage lysins have been reported to reduce bacterial pathogens by > 3 log on fresh and fresh-cut leafy vegetables. However, the approach is relatively new, and data are presently limited. Potential constraints on commercial application include narrow strain specificity, absence of effects against viruses and parasites, and cost.

- Research on alternative biocides for use in fresh and fresh-cut processing is scarce. Only one reference on novel biocides describing a nanoparticle preparation of silica particles was reviewed. Use of the novel biocide resulted in > 5 log reductions of some human pathogens on cut lettuce leaves. Trisodium phosphate was also very effective against some bacteria and viruses. Reasons for the scarcity of research on alternative biocides for use in fresh and fresh-cut processing are unclear; perhaps barriers to regulatory approval contribute to this situation.

Berries and tropical fruits

- Specifically, limited papers are available on the mitigation of protozoa on berries, while several papers exist on virus mitigation. Information is mostly on strawberries, blueberries and raspberries, but it is uncertain how these data may translate to other berries (especially on a global scale). Publications on mitigation efforts for tropical fruits are less common than for berries.
- Water-assisted light treatments (e.g. UV, pulsed light) resulted in > 4 to 5 log reductions in some situations; however, the efficacy depends on how the berries are inoculated (e.g. spot vs dip, calyx vs skin). Some of the studies assessed the disinfection effect of process water to avoid cross-contamination. Ultrasound treatment in combination with a biocide showed some efficacy, 2 to 3 log reductions in some situations, although some adverse effects on product quality were reported, such as reduced firmness in strawberries.
- Gaseous treatments (e.g. controlled-release pads, fumigation, fogging with chlorine dioxide or sulfur dioxide) had variable effects depending on the dose and pathogen assessed.

Melons and tree fruits

- The most important strategy for improving the safety of melons and tree fruits involves hygienic handling and hygiene control including environmental monitoring during the sorting and packing of these products. Keeping the packing environment and packaging equipment free from contamination is essential to reducing risks.
- Water management is a key strategy to maintain the microbiological quality of process water and prevent cross-contamination through the use of biocides or ultraviolet C (UV-C) light treatments of the water.
- There are many decontamination treatments currently available or in the research phase that aim to reduce the levels of pathogenic microorganisms on the surface of melons and tree fruits. However, the degree of reduction

that can be expected from these technologies when applied by the industry is relatively low and will be affected by the characteristics of the rind or surface of the fruit as well as by many other factors. The degree of contamination reduction achieved is typically low. The treatment most commonly found in the literature are UV-C light (e.g. 254 nm, 11 kJ/m^2) and heat (e.g. 65 to 80 °C applied for times from 45 s to 5 min), which generally achieve 1 to 2 log reductions.

- Specific for pome fruit, the use of gaseous biocides in the atmosphere during prolonged refrigerated storage (e.g. controlled atmosphere of low oxygen and ultra-low oxygen) of selected fruits (i.e. pome) was a critical and effective intervention.

Seeded and root vegetables

- As previously stated for other commodities, irradiation is the most effective post-harvest treatment against human pathogens on seeded and root vegetables. Irradiation doses were sufficient for 3 to 5 log inactivation of *Salmonella* on green onions, baby carrots and grape tomatoes. Treatment was not detrimental to quality and was able to slightly extend shelf-life. As previously noted, limitations exist with the availability of technology and consumer acceptance.
- Gas phase chlorine dioxide (ClO_2) treatment has shown efficacy ranging from 2 to 5 log reduction on vegetables contaminated with human pathogens. In-package aerosolized ClO_2 (400 ppm) reduced populations of human pathogens in the stem scar area of tomatoes and lower populations of human pathogens on washed carrots by 2 log units.
- Ultraviolet C shows promise for bacterial surface decontamination of vegetables (approximately 2 log reduction compared to controls) with evidence for inactivation on multiple crops (e.g. tomatoes, cucumber, jalapeno pepper). Crops with greater shadowing or porosity will have less efficacy. Integrated treatment using a low dose of UV-C light with biocides (e.g. organic acids [1 percent], hydrogen peroxide [3 percent], and a novel antimicrobial preparation containing hydrogen peroxide, ethylenediaminetetraacetic acid (EDTA) and nisin) provided a greater than 4 log reduction in *Salmonella* populations on tomatoes.
- Delivery of biocides can be improved by physical means. Incorporation of vacuum impregnation into a washing process (with 2 percent malic acid) reduced levels of human pathogens on paprika, peppers and carrots. The extended processing time and necessity to make this a batch process will be drawbacks to commercial applications.

1

Introduction

1.1 BACKGROUND

Fresh fruits and vegetables are an important part of a healthy diet and are protective against many chronic health conditions. Yet, fresh fruits and vegetables are increasingly being implicated in food safety incidents involving microbiological hazards around the globe. Fresh produce contaminated with foodborne pathogens (bacteria, viruses, protozoa, helminths, etc.) have resulted in numerous outbreaks of foodborne illness and trade disruptions.

The Codex Alimentarius Commission (CAC) initially developed the "Code of Hygienic Practice for Fresh Fruits and Vegetables" in 2003 then later revised it in 2010 following a Joint FAO/WHO Meeting on Microbiological Risk Assessment (JEMRA), held in 2008, to address microbiological hazards associated with leafy vegetables and herbs (MRA14). In addition, several commodity specific annexes were added to the code of practice in 2012, 2013 and 2017 (CXC 53-2003).

Subsequently, in 2018, FAO and WHO published the report "Shiga toxin-producing *Escherichia coli* (STEC) and food: attribution, characterization and monitoring" (MRA31) wherein fresh fruits and vegetables were identified as important sources of STEC infection. In 2019, following a request from the Codex Committee on Food Hygiene (CCFH), the CAC approved new work at its 42nd session on the development of guidelines for the control of STEC in leafy greens and in sprouts. More recently, in October 2020, a Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) of *Listeria monocytogenes* in ready-to-eat (RTE) foods noted increased reports of listeriosis linked to fresh and minimally processed fruits and vegetables.

To meet the request of the CCFH and to update and expand the information available in the previous report "Microbiological Hazards in Fresh Leafy Vegetables and Herbs" (MRA14), FAO and WHO held a series of expert meetings on preventing and controlling microbiological hazards in fresh fruits and vegetables. The goal of these meetings was to gather recent data, evidence and scientific opinions on the topic.

1.2 OBJECTIVES

The purpose of the JEMRA meeting was to collect, review and discuss measures for the control of microbiological hazards during primary production, harvest, post-harvest handling and processing of fresh fruits and vegetables. Emphasis was placed on the identification and evaluation of preventive measures to reduce foodborne illnesses associated with fruits and vegetables, taking into consideration their effectiveness and practicalities.

The objectives of the meeting included the following:

- Identify and characterize microbiological hazards associated with fruits and vegetables, including the main pathogens of concerns, their potential sources and routes of contamination.
- Review mitigation and intervention measures being used at different stages and assess their effectiveness at reducing microbiological hazards.
- Review publicly available literature, guidelines from competent authorities and industry associations (e.g. compliance guidelines, code of practices, etc.) to assess the current state of the knowledge in controlling microbiological hazards in leafy vegetables.
- Respond to CCFH questions (Annex 1).

2

Scope and definitions

2.1 SCOPE

Fresh, ready-to-eat or minimally processed fruits and vegetables are a leading cause of foodborne infections associated with the consumption of fresh produce. This report describes existing interventions or current research regarding interventions meant to reduce the risk of contamination with foodborne human pathogens during the preharvest (primary production) and post-harvest stages in the production of fruits and vegetables.

This report covers risk reduction measures specific to the primary production, harvest, post-harvest handling and processing of fruit and vegetables.

2.2 DEFINITIONS

Fresh (fruits and vegetables): These are fruits and vegetables that are not processed in a manner that changes their physical properties. Cooked, canned, juiced, frozen, candied, dried, pickled, fermented or otherwise preserved foods derived from fruits and vegetables were excluded from this definition and this report.

Ready-to-eat (fruits and vegetables, including minimally processed): These are fruits and vegetables intended for direct human consumption without any additional steps or action taken to reduce or eliminate microbial contamination (modified from FAO and WHO, 2017).

Minimally processed (fruits and vegetables): These are fruits and vegetables that have undergone processes that do not affect their fresh-like quality such as washing, trimming and cutting (modified from FAO, 2020). Fruits and vegetables that are peeled, cut into pieces, chopped, frozen or dried, with the exception of leafy green vegetables, are not included in this report.

STEC (Shiga toxigenic *E. coli*): These are strains of the bacterium *Escherichia coli* that produce Shiga toxin, a potent cytotoxin in humans.

3

Leafy vegetables and herbs

3.1 OVERVIEW OF LEAFY VEGETABLES AND HERBS

Highly diverse production systems (open field, closed, conventional, organic, peri-urban, urban, and less-defined systems) are used to grow a wide range of leafy vegetables worldwide in geographic regions with variable environments, biodiversity, and climate subject to extreme events and changing patterns due to the climate crisis. Moreover, crops derived from these systems are delivered to consumers through varied market channels adapted to a range of cultural practices, consumption patterns, regulatory frameworks and transportation systems. Consequently, there is no prototypical production and distribution scheme for leafy vegetables, and some microbiological hazards are undoubtedly unique to commodities in specific supply chains.

3.2 MICROBIOLOGICAL HAZARDS IN LEAFY VEGETABLES

The presence of robust food safety surveillance systems in high-income countries (HICs) enables the monitoring of microbiological hazards in foodstuffs, effective trace back and epidemiological studies required for source attribution and the identification of foodborne illness outbreaks caused by such hazards. Analysis of data collected in HICs worldwide has shown that leafy vegetables are a leading cause of foodborne infections caused by bacterial, viral or parasitic microorganisms in several jurisdictions. In contrast, the lack of such surveillance systems in low- and middle-income countries (LMICs) means that the prevalence of microbiological hazards in the food supply is not well documented and that the sources of foodborne outbreaks are often not formally identified. However, alternative data sources (for example, records maintained by local public health authorities, medical practitioners, clinicians or hospitals, research reports, etc.) support the notion that leafy vegetables also contribute significantly to the burden of foodborne illness in LMICs.

A wide range of bacterial, viral and parasitic human pathogens may cause outbreaks of foodborne illness transmitted by fresh produce. Analysis of North American and European data collected since 2000 showed that *Cryptosporidium* spp. (20.5 percent) in Europe and *Salmonella enterica* (52.2 percent) in North America caused outbreaks at the highest frequency, and that norovirus (54.3 percent) and *S. enterica* (61.3 percent) were associated with the highest number of cases in Europe and North America, respectively (Aiyedun *et al.*, 2021). All of these pathogens have also been reported as the cause of outbreaks associated with leafy vegetables, although at variable frequencies. For example, the data in the United States of America for the period 1973–2012 showed that norovirus was responsible for 55 percent of leafy vegetables outbreaks with confirmed etiology, and *S. enterica* for 11 percent (Herman, Hall and Gould, 2015). Moreover, STEC from both O157 and non-O157 serogroups, which caused comparatively few outbreaks linked to other fresh produce commodities, were responsible for 18 percent of outbreaks in the United States of America linked to leafy vegetables. Leafy vegetables are currently considered to be a major source of STEC infections in the United States of America (Marshall *et al.*, 2020). Other types of pathogenic *E. coli* , *Listeria monocytogenes, Shigella* spp., *Yersinia enterocolitica* and *Y. pseudotuberculosis, Campylobacter* spp., *Legionella* spp., *Staphylococcus aureus, Leptospira* spp., *Klebsiella pneumoniae* and *K. aerogenes, Cronobacter* spp., *Vibrio parahaemolyticus, V. cholera* and *V. vulnificus, Aeromonas hydrophila* and *A. sobria,* and *Enterobacter cloacae* have also been reported to cause sporadic outbreaks linked to leafy vegetables.

While norovirus is clearly a leading cause of viral foodborne illness transmitted by leafy vegetables, their role in the transmission of other foodborne viral pathogens is poorly understood. Several viruses have been detected in commercial products, including hepatitis A, nipah virus, rotavirus, enterovirus, adenovirus, astrovirus, aichivirus, and sapovirus (Shin *et al.*, 2019; Cuevas-Ferrando *et al.*, 2021). Similarly, epidemiological evidence has confirmed the role of leafy vegetables in the transmission of infections caused by *Cryptosporidium* spp. while comparatively little is known about their role in the transmission of other parasitic species such as Cyclospora *cayatenensis, Giardia lamblia* and *G. duodenalis, Toxoplasma gondii, Angiostrongylus cantonensis* and *A. costarrisensis, Echinococcus multilocularis,* *Ascaris lumbrecoides, Trypanosoma cruvy, Ancylostoma duodenale, Entamoeba* spp., *Balantidium coli, Cystoisospora belli, Blastocystis* spp. and *Enterocytozoon bieneusi.*

3.3 MITIGATION AND INTERVENTION MEASURES EVALUATED WITH LEAFY VEGETABLES

Contamination with bacterial, viral or parasitic microbiological hazards and the proliferation of bacterial species capable of growth outside animal hosts may occur during the cultivation, harvest, post-harvest handling, processing, storage, distribution or consumer handling of leafy vegetables. Consequently, control measures that prevent contamination, inactivate microbiological hazards or restrict the growth of bacterial pathogens before, during or after harvest are needed to reduce risks to consumers. However, several constraints affect the selection of measures intended to ensure the safety of leafy vegetables. Notably, some of the more widely grown and consumed commodities (e.g. lettuce) are primarily eaten raw and have limited shelf-life.

3.3.1 Preharvest applications

Soil and irrigation water are known to be primary sources of hazardous microbiological contaminants in the production environment. Treatment of soil amendments such as animal fertilizers by physical or biological means is known to inactivate a wide range of potential foodborne pathogens that could be transferred to growing crops. Composting, a widely practiced treatment for animal fertilizers, has been shown to reduce the transfer of *E. coli* from amended soil to growing lettuce plants (Chukwu *et al.,* 2022). Treatment of soil amendments is mandated or recommended for leafy vegetable production in many jurisdictions. Additional control may be achieved by the use of physical barriers to prevent contact between soil and edible portions of a growing plant. For example, plastic mulch was shown to reduce the transfer of *Salmonella* from contaminated soil to growing lettuce in laboratory scale experiments (Honjoh *et al.,* 2014). The latter hints that cultivation of leafy vegetables in plastic mulch beds, a common agronomic strategy in some production systems, may contribute to risk reduction.

Interventions aimed at the control of microbiological hazards in soil are generally applied once, at the start of a production cycle. In contrast, the control of microbiological hazards in irrigation water must be exercised over a complete production cycle, during which large volumes of water may be applied to the crop. In the absence of a consistent, reliable supply of irrigation water, treatment to inactivate potential hazards is desirable. Evidently, practical irrigation water treatments should be scalable to accommodate small to large production systems. Experimental irrigation water treatment by UV or with filtration-based systems employing zero-valent iron sand has been shown to reduce populations of bacterial pathogens by up to 6 log in irrigation water, and to significantly reduce the risk of transfer to growing leafy vegetable plants (Beauvais *et al.,* 2021; Marik *et al.,* 2019; Ingram *et al.,* 2012). Several practical considerations may hamper application of these technologies however, including access to power in more remote field settings, controlling flow rates, and the cost of the technologies.

Some attempts have been made to develop treatments that inactivate microbiological hazards on growing plants using chemical or biological agents. Application of the antimicrobial compounds chitosan and tea tree oil acid had little effect against *E. coli* O157:H7 on growing lettuce (Goñi, 2014). However, an acetic acid-based spray treatment applied 1 day prior to harvest was shown to affect the prevalence of *E. coli* O157:H7 or *Salmonella* on leaf lettuce, spinach, or cabbage, although the effect was variable and depended on the type of leafy green (Erickson *et al.,* 2019). Treatment of spinach plants with *Bacillus* spp. reduced *Salmonella* by 1 log (Zhao, 2021), and lactic acid bacteria applied electrostatically within the first 4 weeks of the growing cycle were shown to reduce *E. coli* O157:H7 by nearly 3 log (Laury-Shaw *et al.,* 2019).

3.3.2 Post-harvest applications

The availability of treatments for the disinfection of leafy vegetable plants immediately after harvest would find value both for food safety enhancement in commodities distributed in an unprocessed format, and for the control of microbiological hazards in raw materials destined for minimal processing. Washing in ozonated water has been examined for the control of *Salmonella* on green leaf lettuce heads. While *Salmonella* inactivation was observed in process water, the treatment had no effect on the prevalence of the pathogen on the lettuce tissues (Xu and Wu, 2014). An attempt was made to develop an in-package cold plasma treatment for the control of *E. coli* O157:H7 on bulk romaine lettuce inside a commercial plastic clamshell container. Using this approach, reduction in *E. coli* O157:H7 was limited to 1.1 log (Min *et al.,* 2017). Hence, it appears that there has been little progress in the development of treatments that can reliably disinfect whole leafy vegetable plants.

Several approaches are under consideration for the control of microbiological hazards in leafy vegetables during minimal processing. Some rely on the application of a single physical process or biocide for the inactivation of microbiological hazards, while others combine two or more to enhance the efficacy of the treatments. The following provides a summary of progress in the development of approaches examined to date.

3.3.2.1 Natural antimicrobials applied as dips, sprays, coatings, and packaging films

Numerous natural antimicrobial compounds (NACs) are under consideration for the development of interventions against foodborne pathogens during or after minimal processing of fruits and vegetables. Research is primarily focused on the antimicrobial activity of plant-based NACs, including a wide range of crude extracts, essential oils obtained by distillation, or highly purified compounds recovered from various plants or plant parts. Given the intense sensory character and potential phytotoxicity of some plant extracts, effects on sensory, visual and textural quality are also crucial considerations in the assessment of potential interventions applicable to minimally processed leafy vegetables. Applications considered to date have primarily consisted of direct immersion of the commodity in disinfecting "dips" applied during or after washing, application in the form of sprays or coatings after washing, or incorporation into packaging for release onto the product during storage and distribution. The scientific literature in this research area is extensive. A few examples are presented in Table 1 to illustrate the range of potential applications and commodities that have been investigated to date. Overall, the findings from these investigations show that NACs can inactivate enteric bacterial foodborne pathogens at various stages of processing and on a variety of leafy vegetables. However, the reported antimicrobial activity of some NACs is muted, and there is variability in efficacy reported in different studies or on diverse commodities. In addition, there is a dearth of data on antiviral or antiparasitic effects, and a lack of solubility has necessitated the development of means to improve the functionality of many NACs of interest such as essential oils, by emulsification for example. While effects on product quality are generally assessed and often reported to be slight to non-existent, it must be stressed that such conclusions are derived from laboratory-scale studies. To the best of the experts' knowledge, no plant-based NACs are currently being used in the commercial production of minimally processed leafy vegetables. Consequently, there is a lack of realistic and practical data, knowledge and experience about their impact on the quality of products delivered through commercial processes and distribution channels.

Table 1. Examples of experimental outcomes from research on the inactivation of foodborne pathogens by numerous natural antimicrobial compounds (NACs) from plants in minimally processed leafy vegetables

Natural antimicrobial	**Target product and treatment**	**Experimental outcome**	**References**
Oregano oil nanoemulsion	Fresh-cut lettuce; dipped in 0.05% nanoemulsion for 1 min	3.44 log reductions in *L. monocytogenes*, 2.31 log in *S.* Typhimurium, and 3.05 log in *E. coli* O157:H7	O'Beirne *et al.,* 2015
Oregano oil	Fresh-cut lettuce; dipped in 25, 40 and 75 ppm for 5, 10, 15 and 20 min	Maximum 1.92 log reduction in *S.* Typhimurium	Gündüz, Gönül and Karapınar, 2010a
Oregano, clove, zataria oil emulsions	Baby leaf lettuce; applied by spray	3.5 and 0.5 log reductions in *E. coli* O157:H7 after 5 days at 7 °C after treatment with zataria and oregano, respectively; clove oil was ineffective.	Azizkhani *et al.,* 2013
Cinnamon leaf oil	Spinach, romaine, iceberg lettuce; dipped in 0.1, 0.3, and 0.5% v/v for up to 2 min	Concentration, time and commodity dependent effects against *S.* Newport during subsequent storage at 8 °C	Todd *et al.,* 2013
Cinnamon leaf oil emulsion	Kale; applied in a wash	Immediate 1.83 and 1.54 log reductions against *L. monocytogenes* and *E. coli* O157:H7	Kang *et al.,* 2019
Basil and mint essential oils	Lettuce and purslane; dipped in 0.01 ml/L, 0.032 ml/L or 0.08 ml/L mint for 10 or 15 min	Slight reductions in *E. coli* O157:H7 and *S.* Typhimurium populations during refrigerated storage at 4 °C. Mint essential oil was most effective.	Karagözlü, Ergönül and Özcan, 2019
Clove bud essential oil emulsion	Pak choi; sprayed with 0.02% clove bud oil + 0.002% benzethonium chloride	1.97 and 2.00 log reductions in *S.* Typhimurium and *L. monocytogenes,* respectively	Park, Kang and Song, 2019
Nanoemulsified carvacrol	Baby spinach, romaine, iceberg lettuce; dipped in 0.25 or 0.75% for 2 min	Treatment with 0.75% immediately reduced *E. coli* O157:H7 by 1.3 log on romaine lettuce, 2.3 log after 14 days at 10 °C	Chen *et al.,* 2021

Natural antimicrobial	**Target product and treatment**	**Experimental outcome**	**References**
Cinnamaldehyde and carvacrol essential oils	Basil, cilantro, dill, parsley, tarragon; dipped in cinnamaldehyde (0.3 and 0.5%) and carvacrol (0.1 and 0.3%)	Commodity- and concentration-dependent on effects on bactericidal activity; 5 log reduction in *E. coli* O157:H7 and *Salmonella* on cilantro and dill with 0.3% carvacrol, or 0.5% cinnamaldehyde; Bactericidal effects continued during storage at 4 °C	Patel, Keelara and Green, 2018
Cinnamaldehyde with Tween, Sporan® or acetic acid	Romaine and iceberg lettuce; dipped in 800 and 1 000 ppm cinnamaldehyde and Sporan® alone or in combination with 200 ppm acetic acid	2.89 log reduction in *E. coli* O157:H7 on iceberg lettuce dipped in 800 ppm cinnamaldehyde-Tween	Yossa *et al.*, 2013
Honeybush ethanol extract	Swiss chard; washing in 6 mg/L solution	2.31–2.67 log reductions *L. monocytogenes* and *E. coli* O157:H7	Kang and Song, 2021
Peanut skin extract/ benzethonium chloride emulsion	Romaine lettuce; washing in 5 mg/mL	3.06 and 2.83 log reductions in *L. monocytogenes* and *E. coli* O157:H7	Lee *et al.*, 2021
Polypropylene film with oregano essential oil or citral	Mixed leafy vegetable salad; film containing up to 5%	Release during storage inhibited the growth of *E. coli* , *Salmonella enterica* and *Listeria monocytogenes* at abusive temperatures	Muriel-Galet *et al.*, 2012
Edible films containing carvacrol and cinnamaldehyde	Romaine and iceberg lettuce; in sealed plastic bags made from carvacrol or cinnamaldehyde containing films made from apple, carrot, and hibiscus	5 log reduction in *E. coli* O157:H7, Romaine lettuce placed in 3% carvacrol-containing film	Zhu *et al.*, 2020

3.3.2.2 Modified atmospheres and gas phase antimicrobials

Modified atmosphere packaging (MAP) is widely used to improve the shelf-life, sensory, appearance and quality of minimally processed leafy vegetables. The effects of various packaging films or gas mixtures used in commercial MAP systems on the behaviour of foodborne pathogens appears to be variable. *E. coli* O157:H7 was shown to grow in shredded cabbage stored at 10 °C in air, under 5 percent, 10 percent and 15 percent CO_2, and in MAP systems designed to generate oxygen-depleted atmospheres using either high- or low-oxygen transmission permeability films (Izumi and Inoue, 2018). Oxygen-depleted atmospheres (0.25 percent O_2 + 12 percent CO_2 and 2 percent O_2 + 6 percent CO_2) were reported to stimulate the growth of *L. monocytogenes* on fresh-cut Iceberg lettuce (O'Beirne *et al.,* 2015). In contrast, composition of the atmospheres in packages made with films of different permeability had no significant effect on the survival and growth of *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* in shredded lettuce stored at 5° C (Oliveira *et al.,* 2010). Consequently, it appears unlikely that MAP or other systems designed to alter atmospheric gas composition in leafy vegetable packaging systems can be relied upon to control human pathogens. In contrast, exposure to antimicrobials in the gas phase has been shown to inactivate foodborne pathogens. At lab scale, treatment with 10 percent hydrogen peroxide for 10 min was shown to reduce *S.* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes* populations on lettuce by 3.12, 3.15 and 2.95 log per g, respectively (Back, Ha and Kang, 2014). However, these results should be taken with caution and cannot be directly extrapolated to industry scale. The potential use of gaseous ozone for the disinfection of leafy vegetables also remains to be fully explored (Fan, 2021). In all cases, applications in leafy vegetable processing would require the design of equipment to accommodate small to large commercial operations that operate at very high line speeds.

3.3.2.3 Irradiation

Irradiation by gamma ray, electron beam or X-ray is well known to inactivate microorganisms in foodstuffs. Consistent reductions in bacterial foodborne pathogens reaching 5–6 log in leafy vegetables have been reported, usually without negative effects on quality (Gobeil, Shankar and Lacroix, 2020; Mahmoud, Bachman and Linton, 2010; Moosekian, Jeong and Ryser, 2014; Niemira 2008; Niemira and Cooke, 2010; Rezende *et al.,* 2014; Sanglay *et al.,* 2011). Equipment adaptable to the processing of fresh produce is commercially available. Obstacles to the use of irradiation in food processing are not technological and have been dealt with elsewhere (Castell-Perez *et al.,* 2021)

3.3.2.4 Plasma and combinations

Partially ionized molecules generated by applying energy to gases have long been known to have antimicrobial activity. Electrical discharges generated by various means (dielectric barrier, radiofrequency, microwaves, etc.) can be used to generate plasma at temperatures close to ambient, allowing for the treatment of heat-sensitive materials. Because the active antimicrobial species are present in the gas phase, plasmas are also well-suited to the treatment of food contact surfaces. Given these properties, there is considerable interest in the use of plasma technology for the disinfection of fresh produce, including leafy vegetables. Research has shown that treatment with plasma can inactivate enteric bacteria on the surface of leafy vegetables, with reductions ranging from 1.6 log *E. coli* on cut lettuce immediately after treatment (Bermúdez-Aguirre and Barbosa-Cánovas, 2013); to 3.18 and 3.77 log for *Salmonella enterica* and *E. coli* O157:H7 on spinach, measured 14 days in storage after treatment; 2.19 log *E. coli* O157:H7 on lettuce treated with plasma in combination with essential oil of clove (Cui, Ma and Lin, 2016); and up to 5 log *Aeromonas hydrophila* on lettuce (Jahid, Han and Ha, 2014). The treatment can also be applied inside packages. For example, a 1.5 log reduction in *Salmonella* was measured on cabbage slices in polyethylene terephthalate (PET) containers treated with hydrogen peroxide in combination with plasma generated by dielectric barrier discharge (Kim and Min, 2021).

Although it is clear that plasma is a promising technology for the disinfection of fresh produce including leafy vegetables, and that considerable progress has been made in the development of equipment suitable for commercial-scale processing, there is presently little evidence of industrial uptake. A wide range of plasma technologies, commodities and experimental conditions have been employed in the assessment of plasma-based disinfection treatments, leading to considerable variability in experimental outcomes. Consequently, additional research and development are needed to support the transition to commercial applications, notably with respect to the standardization of treatments across commodities and to the cost of the treatment (Asghar *et al.,* 2022).

3.3.2.5 Ultrasound and combinations

Sound waves with frequencies above 20 kHz, referred to as ultrasound, have long been applied in the processing of liquids because they can improve mixing and accelerate chemical reactions. High power and intensity ultrasound alone can disrupt bacterial cell membranes and produce free radicals leading to cellular damage and death. However, such conditions can induce undesirable ultrastructural changes in food matrices, notably in fresh produce where the maintenance of cellular integrity and physiological processes are essential for the extension of shelf-life. Consequently, ultrasound has primarily been applied in combination with other antimicrobial strategies as a means to improve their efficacy. For example, the biocidal effect of sodium hypochlorite solutions against *E. coli* is enhanced by the application of sonication (Duckhouse *et al.,* 2004). Mild sonication was shown to improve the efficacy of chlorine, acidified sodium chlorite, peroxyacetic acid, and acidic electrolyzed water washes by 0.7 to 1.1 log over those measured when washing with sanitizer alone against *E. coli* O157:H7 on spinach, without affecting organoleptic quality (Zhou, Feng and Lou, 2009). Additional work showed that reductions in *E. coli* O157:H7, *Salmonella* Typhimurium and *L. monocytogenes* on lettuce washed in malic, citric or lactic acids were 8.0 to 1.0 log higher when ultrasound was applied during treatment (Sagong *et al.,* 2011), and inactivation of *Salmonella* on lettuce was improved when ultrasound was combined with washing in solutions containing oregano and thyme essential oils (Millan-Sango *et al.,* 2016). Hence, it appears that ultrasound can improve the performance of a range of sanitizers applied during the washing of leafy vegetables.

3.3.2.6 Ultraviolet light and combinations

Ultraviolet (UV) radiation comprises the region of the electromagnetic spectrum between 100–400 nm. The region is further divided into three subregions, including ultraviolet C (UV-C) which includes wavelengths between 100–280 nm. Ultraviolet C has well-known microbicidal properties that are exploited for disinfection in many contexts, including food processing and the control of post-harvest diseases in fruit and vegetable storage. Early attempts to adapt UV-C for the disinfection of leafy vegetables showed that treatments designed to inactivate microorganisms often induce tissue damage affecting overall quality and/or shelf-life (Allende *et al.,* 2006). More recent research has shown that lower intensity UV-C or higher wavelength UV treatments can be combined with other approaches to lessen adverse physiological effects in leafy vegetables. For example, a low intensity UV-C treatment combined with peracetic acid reduced *Salmonella* on shredded iceberg lettuce by 3.24 log (Lippman *et al.,* 2020). Treatment with aerosolized malic acid and UV-C could reduce *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* by 2.89, 1.38 and 2.95 log on shredded lettuce (Lee *et al.,* 2021; Lee, Kim and Yoon, 2021), and a UVA treatment combined with washing in acetic acid reduced the same pathogens 3.50, 3.29 and 4.30 log in spinach (Jeong and Ha, 2019). No adverse quality effects were found with either treatment, although long treatment times (50 min for shredded lettuce, 90 min for spinach) were needed to obtain the stated reductions.

3.3.2.7 Pulsed light and combinations

Pulsed light consisting of continuous broad-spectrum infrared, visible and ultraviolet radiation applied for short periods of time (typically 100–400 microseconds) has been shown to inactivate foodborne bacteria and viruses in a range of food products, including fresh produce (Salehi, 2022; Jubinville *et al.,* 2022). The light source is commonly a Xenon lamp, which emits radiation from deep UV-C (200 nm) to near-infrared (1 100 nm) wavelengths. Pulsed light treatments are generally applied in chambers engineered for the purpose and can be used with packaged products. Recent work has shown that treatment of unpackaged romaine lettuce can reduce *E. coli* O157:H7 by 2.68 log, and by 2.52, 2.31 and 2.18 log in packages of 0.00254, 0.00508 and 0.00762 cm thickness (Mukhopadhyay *et al.,* 2021). Pulsed light has also been shown to inactivate *Cryptosporidium parvum* oocysts by 2.4, 4.3 and 2.5 log on cilantro, mesclun lettuce, and spinach, respectively (Craighead *et al.,* 2021). Risks of light-induced alterations in the quality of target produce have led to the consideration of combined treatments designed to reduce the intensity or length of exposure during treatment. Using this approach, reductions of > 5 log *E. coli* O157:H7 were measured on spinach when a brief pulsed light treatment was combined with the use of a novel sanitizer consisting of hydrogen peroxide, EDTA and nisin (Mukhopadhyay *et al.,* 2019). While equipment needed for pulsed light processing is available, there is little evidence of industry uptake for the processing of leafy vegetables.

3.3.2.8 Alternative biocides

Little research effort has been directed to the development of novel biocides for use in the minimal processing of fresh produce. One exception concerns a novel biocide consisting of sodium hypochlorite bound to the surfaces of modified silica microparticles which can generate localized high concentrations of chlorine. Using these particles, > 5 log reductions in *E. coli* O157:H7 and *Listeria innocua* populations were achieved during washing of produce, even in the presence of high-organic loads (Huang and Nitin, 2019). Reasons for the slow pace of progress in the development of new, more effective biocides are unclear.

3.3.2.9 Biocontrol methods

Biocontrol strategies employing antimicrobials produced by microorganisms and bacteriophage have been investigated for the control of foodborne bacterial pathogens in leafy vegetable products. Various bacteriocins and peptides including nisin, reuterin, and novel compounds produced by lactic acid bacteria have been shown to either inactivate or slow the growth of the Gram-positive bacterium *L. monocytogenes* in stored, packaged lettuce (Asare *et al.,* 2018; Dong *et al.,* 2021; Randazzo *et al.,* 2009; Yi *et al.,* 2021). In addition, these post-process treatments have little to no effect against Gram-negative foodborne pathogens, which restrict their utility to the control of a single microbiological hazard. In contrast, bacteriophage preparations that target *Salmonella, E. coli* O157:H7 and *L. monocytogenes,* the most common bacterial foodborne pathogens associated with leafy vegetables, are all available commercially. A commercial bacteriophage preparation that targets *E. coli* O157:H7 was shown to reduce populations by 2.5 log on lettuce leaves, thereby reducing the risk of cross-contamination during processing (Ferguson *et al.,* 2013). Using the same bacteriophage preparation, *E. coli* O157:H7 populations were significantly reduced in romaine, green leaf lettuce and spinach stored at 4 °C and 10 °C (Boyacioglu *et al.,* 2013). In addition, a commercial bacteriophage preparation that targets *Salmonella* was shown to reduce populations by 2–3 log, and a preparation that targets *L. monocytogenes* reduced populations from 1 to 2 logs on lettuce leaves and fresh-cut curly endive, respectively (Perera *et al.,* 2015; Truchado *et al.,* 2020; Zhang *et al.,* 2019). These findings suggest that bacteriophage preparations could be useful in the development of interventions that target one or more foodborne pathogen in minimally processed leafy vegetables.

Bacteriophage used in the formulation of commercial preparations inactivate target bacteria through the release of lysins, phage-encoded enzymes that degrade the bacterial cell wall resulting in lysis and death. A recent report describes a novel lysin with potent bactericidal activity against several Gram-negative bacterial pathogens including *E. coli, Salmonella, Shigella* and *Acinetobacter* (Xu *et al.,* 2021). The lysin could inactivate 99.7 percent of *E. coli* O157:H7 on the surface of lettuce leaves. While there is anecdotal evidence of industrial application of bacteriophage in leafy vegetable processing, there remains a lack of available technical data to guide applications in commercial settings.

4

Berries and tropical fruits

4.1 OVERVIEW OF BERRIES AND TROPICAL FRUITS

Berries are a highly perishable food commodity, which is widely consumed without prior treatments to inactivate pathogens. Tropical fruits, like mangoes, may have a longer shelf-life from farm to fork but are largely consumed without further treatment. Examples of berries are strawberries, blueberries, raspberries, blackberries and gooseberries. The class known as tropical fruits includes avocados, mangoes and papayas among many others.

Berries can be produced in open fields and controlled environment agriculture (CEA), which includes indoor agriculture (e.g. greenhouse, low tunnels and net houses) and vertical farming. Plants grown in CEA are usually produced using different types of hydroponic systems, such as drip hydroponics, deep water culture, aeroponic or aquaponic systems among others. The same preventive measures, such as GAP, GHP, and GMP (for enclosed production facilities) apply. In order to prevent bruising or injury, most berries are harvested manually and picked directly into baskets, punnets, boxes or other containers that are further used for distribution and retail. After harvest, the products are typically cooled (to rapidly remove field heat) before transport, distribution and retail. Since berries are prone to mechanical damage and rapid growth of moulds resulting in soft rots, further interventions such as washing are not commonly used. Tropical fruits, considered here, grow mostly above ground on trees or bushes in open fields and CEA. The food safety preventive measures mentioned above apply here as well. These fruits may be harvested mechanically or by hand. Water sprays are commonly applied, and in packing lines, fruits are usually transported in flumes containing biocides. Fruits may be cooled on the farm using hydro-, forced air- or room-cooling methods.

4.2 MITIGATION AND INTERVENTION MEASURES EVALUATED WITH BERRIES

Given the frequency of contamination events, in particular those involving non-bacterial contaminants, it is likely that future research will include studies to enhance mitigation strategies directed to protozoa and viruses. Data gaps and uncertainty regarding commercialization of mitigation strategies are evident in the review of available data.

4.2.1 Post-harvest applications

Several interventions have been tested on berries focused on the removal and inactivation of bacterial pathogens, e.g. different kinds of washing procedures, gaseous treatments and films and coatings. A general observation for most of the studies is that they have been carried out in experimental or small scale (from a few berries to 100 g samples) and that testing in more industrial settings is lacking. Information is mostly on strawberries, blueberries, raspberries, and bacterial pathogens, while there is limited information available on mitigation of protozoa on berries. Several papers exist on virus mitigation. It is uncertain how the existing data may translate to other berries (especially on a global scale).

4.2.1.1 Water-assisted ultraviolet and pulsed light treatments

Water-assisted light treatments (e.g. UV, pulsed light) have been tested alone or in combination with biocides. A combination of water-assisted pulsed light treatment and H_2O_2 resulted in 4.0 to > 5.5 log CFU/g reduction of *Salmonella* for raspberries and blueberries, respectively. No viable bacterial cells were recovered from process water after this treatment even in the presence of a high-organic load (Huang *et al.*, 2015).

Other authors have reported different efficacies for water-assisted pulsed light treatments depending on how the berries were inoculated. Blueberries spot inoculated on skin, followed by calyx inoculation, showed the largest reductions of *Salmonella* and *E. coli* O157:H7 compared to dip inoculation which resulted in the lowest reductions. No significant improvement of shelf-life was observed (Cao, Hang and Chen, 2017; Huang and Chen, 2014).

In another study, water-assisted pulsed light decontamination was compared with water-assisted UV light decontamination against *Salmonella* in blueberries. Depending on inoculation method, water-assisted pulsed light and water-assisted UV reduced numbers of *Salmonella* by approximately 5 log CFU/g (4.5 to > 5.6 log CFU/g) on berries that were calyx- or stem-inoculated, and 2 log CFU/g for berries that were dipinoculated. Viable *Salmonella* were recovered from the process water after the treatments, and the authors recommended including a biocide to prevent cross-contamination through the process water (Huang and Chen, 2019).

Testing of water-assisted UV light indicated reductions of *Salmonella* and *E. coli* O157:H7 in the same range as for water-assisted pulsed light with a similar dependency on inoculation method (Huang and Chen, 2020; Liu, Huang and Chen, 2015). For murine norovirus on blueberries the reduction ranged from 1.81 to > 4.3 log PFU/sample depending on inoculation method and scale of experiment (Liu, Huang and Chen, 2015). The results from testing application of water assisted UV-light indicated that increased chemical oxygen demand (COD) in the water reduced the efficacy of decontamination (Huang and Chen, 2020; Liu, Li and Chen, 2015).

4.2.1.2 Ultrasound and combinations

Ultrasound has also been applied in combination with different chemical sanitizers. The reductions varied from approximately 3 log CFU/g for *L. innocua* on blueberries to 2 log CFU/g for *Salmonella* on strawberries (Zhang, Tsai and Tikekar, 2021; do Rosário *et al.,* 2017). However, there were still viable bacteria in the process water. Some adverse effects of ultrasound treatment have been reported. The results from a study on *L. innocua* on blueberries indicated some influence on colour, while low frequency ultrasound significantly reduced the firmness. High frequency ultrasound had little impact on blueberry firmness (Zhang, Tsai and Tikekar, 2021). Another study reported reduced firmness of strawberries with treatments that included ultrasound, while reporting a variable reduction of aerobic mesophiles and yeast and moulds (de Sao José and Vanetti, 2015). Other studies have reported little loss in sensory quality, with 1–2 log CFU/g reductions of *Salmonella* and *E. coli* O157:H7 in strawberries and blueberries (do Rosário *et al.,* 2017; Wang and Wu, 2022).

4.2.1.3 Hydrocooling and temperature stabilization

After harvest, berries are cooled before storage and distribution. In a study on intact strawberries inoculated with *Salmonella*, forced-air cooling was compared with hydrocooling in water containing 100 or 200 ppm hypochlorous acid (HOCl). Hydrocooling significantly reduced the levels of *Salmonella*, ranging from almost 2 log CFU/berry when hydrocooling with water alone, to more than 4 log CFU/berry reduction when hydrocooling with 200 ppm HOCl. Storage after the initial cooling led to further reductions of *Salmonella* (Sreedharan *et al.,* 2015). In another study looking at the fate of *E. coli* O157:H7 and *Salmonella* on strawberries and blueberries, it was observed that at a storage temperature of 2

°C the populations declined over 7 days under all conditions applied for both strawberries and blueberries. At 15.5 °C numbers of *E. coli* O157:H7 and *Salmonella* declined on strawberries, while on blueberries, the *Salmonella* populations initially declined but increased to a population close to the initial level after 7 days (Nguyen, Friedrich and Danyluk, 2014).

4.2.1.4 Gaseous treatments and coatings

Assessment of surface treatments includes evaluation of coatings and gaseous treatments, of which the use of gaseous treatment with chlorine dioxide may have potential for large-scale production (Malka and Park, 2021). Edible coatings and surface treatments studied include traditional mitigation strategies like silver nanoparticles, sodium alginate, chitosan, and essential oils. These surface treatments provide variable bacterial inactivation against *E. coli* , *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella*. Berries were treated at the post-processing stage prior to storage. Bacterial enumeration across the studies was variable and some studies commented on improved shelf-life correlated with surface treatment.

4.2.1.5 Irradiation, ultraviolet (UV), and pulsed light treatments

A few studies have investigated the use of irradiation or light treatments. Electron-beam inactivation of a norovirus surrogate in strawberries indicated less than 1 log reduction at doses up to 6 kGy with a maximum reduction of approximately 2 log CFU/g at 12 kGy (Sanglay *et al.,* 2011). Blueberries inoculated with *Toxoplasma gondii* oocysts were exposed to low-dose gamma radiation at 4 °C. The results indicated that the viability of the oocysts was significantly reduced after even the lowest level of treatment (0.2 kGy). Immediately after the treatment, no adverse effect was observed on product quality (Lacombe *et al.,* 2017). Two other studies have looked at pulsed light and UV-C inactivation of *E. coli* O157:H7 and *Salmonella* on fresh raspberries and *E. coli* O157:H7 and *L. monocytogenes* on strawberries and raspberries. Variable bacterial inactivation was observed. Both studies indicated that surface characteristics influenced the efficacy of the treatments (Adhikari *et al.,* 2015; Xu and Wu, 2016).

4.3 MITIGATION AND INTERVENTION MEASURES EVALUATED WITH TROPICAL FRUITS

Considerably less information is available for interventions tested on tropical fruits with regard to mitigation efforts, while several studies on pathogen persistence on tropical fruits exist. As observed for berries, the studies carried out have mainly focused on bacterial pathogens such as *Salmonella* and *L. monocytogenes*. Although some studies have included shelf-life and sensory quality studies to some extent, the treatments' effect on these important qualities is often overlooked.

4.3.1 Post-harvest applications

4.3.1.1 Antimicrobial dips and sprays

Different biocides have been tested on whole mangoes, papaya, and Hass avocados. A combined treatment using alkaline electrolyzed oxidizing water and acid electrolyzed oxidizing water reduced numbers of *L. monocytogenes, Salmonella* and *E. coli* O157: with 4–5 log CFU/cm^2 on inoculated Hass avocados (Rodriguez-Garcia, Gonzalez-Romero and Fernandez-Escartin, 2011). Another study indicated that using aqueous ClO_2 (10 ppm) generated with different acids, especially ClO_2 generated with malic acid, reduced numbers of *S.* Typhimurium and *L. monocytogenes* on whole papaya (Dong and Li, 2021). A third study looked at the antibacterial effect of different roselle calyx extracts tested on 11 foodborne bacteria inoculated on whole mangoes. Acetonic, ethanolic and methanolic extracts of roselle calyx resulted in greater reductions in all the foodborne bacteria than the chemical sanitizers tested (sodium hypochlorite, colloidal silver and acetic acid) (Rangel-Vargas *et al.*, 2018).

4.3.1.2 Antimicrobial blue light and irradiation treatment

The antibacterial effect of 405 ± 5 nm light emitting diode illumination has been tested towards *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* on the surface of fresh-cut mango and towards *Salmonella* on fresh-cut papaya (Kim *et al.*, 2017; Kim, Bang and Yuk, 2017). In combination with chilling, the numbers of bacteria were reduced by approximately 1 log CFU/g after treatment of 36–48 hrs. There was no impact on the physiochemical quality of fresh-cut mango after storage. X-ray treatment of whole mangoes inoculated with *E. coli* O157:H7, *L. monocytogenes, Shigella flexneri* and *Salmonella* resulted in reductions from less than 2 log CFU/cm^2 (*L. monocytogenes*) to approx. 5 log CFU/cm^2 (*Salmonella*) using 0.5 kGy (Kim *et al.*, 2017; Mahmoud *et al.*, 2015). The populations of all the foodborne pathogens were reduced to numbers below the detection limit using 1.5 kGy (Mahmoud *et al.*, 2016)

4.3.1.3 Antimicrobial films

The effect of nisin-incorporated cellulose films was tested on minimally processed mangoes inoculated with *L. monocytogenes* and *S. aureus* (Barbosa *et al.,* 2013). After 4 days of storage at 5 °C, populations of *L. monocytogenes* were below the detection limit in the quantitative analyses. For *S. aureus*, a 6 log reduction was observed after 6 days of storage. Similar to previous studies, these results should be taken with caution and cannot be directly extrapolated to industry scale.

5

Melon and tree fruits

5.1 OVERVIEW OF MELONS

It is well known that contamination of melons in the field cannot be avoided entirely and additional barriers can be applied to reduce the prevalence and concentration of pathogens. Numerous intervention strategies have been suggested to efficiently reduce the risk of contamination at the preharvest and harvest stage and during post-harvest handling. When selecting an intervention, it is important to consider the effectiveness of controlling pathogens against economic, legal, and fruit-quality implications that will influence the sanitizer concentration and contact times applied by the industry (Bartlett *et al.*, 2020).

5.2 MITIGATION AND INTERVENTION MEASURES EVALUATED WITH MELONS

5.2.1 Preharvest applications

5.2.1.1 Antimicrobial treatments

Biocides to reduce the concentration of pathogens in melons are mostly applied at the post-harvest stage, but some studies have been reported at the preharvest and harvest stage. This is the case of the use of in-field stem scar injections of 200 µl of 7.5 percent levulinic acid (LVA) with 0.5–1.0 percent sodium dodecyl sulfate (SDS) followed by a spray of 30 ml 7.5 percent LVA with 0.5 percent SDS to prevent *L. monocytogenes* and *S.* Poona contamination from post-harvest through transport to the packhouse (Webb *et al.*, 2015a, 2015b). The pathogens inoculated on field-treated cantaloupe rind exhibited a greater reduction when cantaloupes were treated with LVA/SDS compared to chlorine in the dump tank. However, as previously highlighted by Bartlett *et al.* (2020), the application of biocide sprays and injections in the field to control pathogens is unlikely to be feasible in a commercial setting.

5.2.2 Post-harvest applications

5.2.2.1 Prevention of cross-contamination during washing

Washing of melons has the main objective of removing dirt, dust and soil. Washing can be a dedicated process step or a combination to cool and transport the melons. If water is used in the post-harvest process to wash, cool or transport melons, the initial source water should meet the microbial standards of drinking water. However, after product contact and subsequent reuse of the process water (e.g. when multiple batches of melons are washed in the same tank or in a water recirculating system), microbiological contamination of the process water may occur, resulting in cross-contamination to other melons exposed to the water, redistributing pathogens to a much larger volume of produce. Therefore, the use of biocides during washing is primarily applied to maintain the microbiological quality of process water. The washing system should be adapted based on the organic load of the incoming melons (i.e. melons grown in open field or CEA, dust storm during production). Washing of melons with water allows the physical removal of soil and microorganisms. This is often done with overhead sprays in conjunction with brushing to enhance removal. However, brushing also removes a portion of the natural waxy cuticle on the product surface that acts as a barrier to microorganisms (Gil and Selma, 2006). During washing, any pathogens which may be present on the rind surface may be reduced, but they are unlikely to be eliminated by washing (USFDA, 2013).

Most of the available literature regarding the use of biocides during washing of fruit and vegetables concluded that washing with a residual concentration of a biocide is used to maintain the microbiological quality of the water and does not considerably enhance the reduction of microorganisms on the surface of the produce (Gil *et al.,* 2009). On the other hand, water systems might become a harbourage and a reservoir for *L. monocytogenes.* Maintenance of storage tanks, tubing systems and filtration systems used in water distribution should be performed to avoid biofilm formation and potential *L. monocytogenes* presence (PROFEL, 2020).

While disinfection or decontamination applied to melon rinds can reduce the prevalence and populations of pathogenic microorganisms, interventions that prevent contamination in the packing house, and the potential contamination of fruit due to cross-contact from the environment, appear to be just as important (Bartlett *et al.,* 2020). There are many decontamination treatments currently available that aim to reduce the levels of pathogenic microorganisms on the surface of the product. However, the degree of contamination reduction that can be expected from these technologies when applied by the industry is relatively low and will be affected by the characteristics of the rind of the melons as well as by many other factors.

5.2.2.2 Ultraviolet light

Available scientific evidence shows a large variability among the efficacy of different physical treatments to eliminate pathogens on the surface of melons. In lab-scale studies, UV-C was effective at reducing the microbial load on the melon surface (Terao *et al.,* 2021). However, implementing the treatment at a commercial scale seems to be difficult because a homogenous application of the treatment for all surfaces of the product has technological limitations.

5.2.2.3 Heat

Heat treatments seem to be a promising intervention strategy to reduce contamination of melons. Lab-scale studies reported large microbial reductions after the use of thermal treatment on melons (Selma *et al.,* 2008; Ukuku, 2006), and these results have been supported by some studies performed at pilot scale (Bezanson *et al.,* 2018). The study published by Bezanson *et al.* (2018) concludes that steam sanitization provides an effective means for the control of pathogen and spoilage organisms, but proliferation of microorganisms on heated cantaloupes was observed during storage, which raises concern regarding the impact of post-processing contamination on consumer health risk. Few research studies evaluate the microbial reduction on the melon rinds after heat treatment. However, studies done in the natural microbiota or spoilage microorganisms indicated that a thermal water immersion of fresh melons can result in a 3 log reduction of surface contamination (Fouladkhah and Avens, 2010). Bartlett *et al.* (2020) summarized the relevant information previously published in two studies (Suslow and Callejas, 2015; Ukuku *et al.* 2016), indicating that the use of heat treatment (65–80˚C applied for times from 45 s to 5 min) managed a reduction of *L. monocytogenes* and *L. innocua* greater than 3 log CFU on the surface of rock melons. However, there are few studies investigating the potential of hot water treatments alone as a sanitization treatment. Based on conclusions made by Bartlett *et al.* (2020), there is also an increased risk of recontamination following the treatment that is not well understood.

5.2.2.4 Antimicrobial dips and sprays

Many research papers have been published evidencing the efficacy of chlorine (50–200 ppm) to reduce the contamination of pathogenic bacteria in the rinds of the melons (Araya-Rodriguez *et al.*, 2008; Svoboda *et al.*, 2016; Ukuku, Mukhopadhyay and Olanya, 2018). In a very comprehensive review of the efficacy of chemical treatments, Bartlett *et al.* (2020) concluded that several studies have demonstrated the effectiveness of chlorine and other biocides to reduce *L. monocytogenes* and other pathogens on the surface of whole melons. However, log CFU reductions of > 3 for *L. monocytogenes* on the surface of melons were not achieved in any study that assessed chlorine at 100 or 200 ppm for a 2 min contact time. In general, the available studies indicate that 100 ppm or 200 ppm concentrations for 2 min at ambient temperature can achieve a < 2 log reduction in pathogenic microorganisms on the surface of whole melons. In studies mimicking industrial conditions, the observed reductions are usually very low, while those studies using more artificial experimental designs show the greatest reductions (Trinetta, Linton and Morgan, 2013).

The same trends observed for chlorine have been reported for other decontamination agents such as hydrogen peroxide, chlorine dioxide, peroxyacetic acid, ozone, and quaternary ammonium (Svoboda *et al.*, 2016). Bartlett *et al.* (2020) concluded that there is insufficient research to confidently recommend an optimal contact time for sanitizers. However, standard recommendations including free chlorine (15–20 ppm), peroxyacetic acid (80 ppm), and chlorine dioxide (aqueous; 3 ppm) for contact times up to 2 min could be applied. Levulinic acid (LVA) and sodium dodecyl sulfate (SDS) have been applied as a post-harvest decontamination treatment to whole melons (Webb *et al.* 2015b). In these studies, different inoculum sizes and combinations of treatments were applied, but the most efficient treatment (2.5 percent LVA/2.5 percent SDS) reduced initial populations of *S.* Poona (4.26–5.04 log CFU/sample) on rind tissue to levels only detectable by culture enrichment when cantaloupes were subsequently exposed to the LVA/SDS solution. The efficacy of the treatments was compared by the authors to a simulated commercial dump tank treatment incorporating 200 ppm chlorine.

5.2.2.5 Natural antimicrobials applied as dips, sprays and coatings

There are many research studies that focus on the use of natural compounds and extracts as antimicrobial compounds to reduce contamination on melons. However, these studies are performed using high inoculum levels and high concentration of the extract, which in many cases affect the sensory qualities of the final product (e.g. flavour, appearance, odour). Although the microbial reductions reported by these studies are in most of the cases very significant (> 3 log units), the suitability of these treatments under commercial conditions is not well understood.

Lactic acid as well as other organic acids are often used as bio-preservatives and have been extensively investigated as processing aids for fresh produce (Bartlett *et al.*, 2020). Examples of research studies focused on the use of organic acids include the study of Singh, Hung and Qi (2018) who evaluated the efficacy of 2 percent lactic acid wash against *L. monocytogenes* and *Salmonella* on the surface of whole rock melons. Kang and Kang (2017) evaluated the antimicrobial effect of vacuum impregnation (VI) applied with 2 percent malic acid against *Salmonella* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes* on muskmelons, showing that after 20 min of VI treatment, population decrease of the three pathogens ranged from 2 to 3 log CFU/cm^2. In general, although some organic acids demonstrated a > 3 log CFU reduction of different pathogens, which indicates that acid washes may have an application as part of hurdle technology, further studies are needed to confirm the suitability of these treatments (Bartlett *et al.*, 2020).

Many research studies focus on the use of essential oils as potential replacements for other chemicals to reduce microbial contamination in melons, which lately have included the development of essential oil nanoemulsions (Bartlett *et al.*, 2020). Most of these studies evaluate the essential oil's efficacy using experimental designs that do not mimic industrial conditions. Therefore, most of the results obtained from available research articles do not demonstrate to industry that they are effective. In the future, research done under more realistic conditions is necessary to confidently specify recommendations for optimal application.

The most commonly used post-harvest fungicide (i.e. imazalil) is now banned in Europe, and this will favour the application of edible coatings to avoid spoilage of the fruit, which might have an impact on foodborne pathogens. Edible coatings are being explored utilizing polysaccharides as coating materials (e.g. alginate or chitosan).

5.2.2.6 Biocontrol methods

Several biological decontamination treatments meet regulatory requirements for use on food, such as bacteriophages and bacterial protective cultures. The efficacy of these post-process treatments in reducing the microbial load of food has been mostly investigated in food of animal origin, particularly on ready-to-eat (RTE) meat and cheese products. Previous studies have shown very promising results, but optimization of the use of these treatments should be done under commercial conditions on specific fresh products to get insight into their real potential.

The use of bacteriocin-producing (Bac+) strains of lactic acid bacteria (LAB) either singly or in combination as protective cultures has been proven to be an efficient method for the reduction of specific pathogenic bacteria on melons. For instance, Tran *et al.* (2020) demonstrated that *Bacillus amyloliquefaciens* ALB65 is an effective biological control agent for the reduction of *L. monocytogenes* growth on intact cantaloupe melons under both pre- and post-harvest conditions. However, it should be taken into account that the reported bacteriocin-producing strains exerted a minimal control over the growth of the pathogen when tested on naturally contaminated vegetables.

In 2016, Bai *et al.* defined bacteriophages as the next-generation biocontrol agents due to the potential of single phage and phage cocktail treatments to control various foodborne pathogens, and bacteriophages can be used as an alternative for conventional food preservatives. Specifically, in fresh produce, 2 log reductions in the level of *L. monocytogenes* have been reported (Oliveira *et al.*, 2014). Leverentz *et al.* (2003) evaluated the use of phages to inactivate *L. monocytogenes* on melons. These authors obtained reductions of about 2.0–4.6 log CFU per sample. In general, application of 6.0–8.0 log PFU/g or ml phage significantly ($p < 0.05$) reduced *L. monocytogenes* populations inoculated on fresh produce (EFSA Panel on Biological Hazards [BIOHAZ], 2016). However, most of the studies were conducted using lab-scale experiments, and thus, results are difficult to extrapolate to the industrial conditions.

5.2.2.7 Edible coatings

Edible antimicrobial coatings or films to extend the shelf-life and deliver antimicrobials to fresh produce are being evaluated. Commercial edible coatings are currently being used in the fresh produce industry to increase shelf-life of products, but their use specifically as a control for foodborne pathogens has garnered a lot of interest (Bartlett *et al.*, 2020).

Several studies are available on the efficacy of edible coatings as an intervention strategy to reduce microbial contamination in melons up to 5 log CFU. Zhang *et al.* (2020) showed that the use of cellulose nanofiber (CNF)-based coating containing chitosan (CHI, 1 percent) and trans-cinnamaldehyde (TC, 1 percent) was effective in eliminating *S. enterica* and *E. coli* O157:H7 inoculated at densities of 6–6.2 log (CFU/cm^2). After evaluating several studies on the use of coatings as an antimicrobial strategy, Bartlett *et al.* (2020) concluded that antimicrobial coatings have the advantage of prolonging exposure to the antimicrobial, but it is important to determine whether antimicrobial coatings actually inactivate pathogens or only suppress growth. They also revealed that, although large reductions of pathogens were reported for specific coatings, the methods of enumeration in the studies may not be sufficiently rigorous to rule out that pathogens were, in fact, not inactivated on the surface of melons rather than being removed with the film, trapped under the film, or suppressed on growth media by active ingredients that were transferred from the coating during the sampling and enumeration procedure (Bartlett *et al.,* 2020). Future research will need to confirm the effectiveness of antimicrobial coatings before they can be effectively applied by the industry.

5.3 OVERVIEW OF POME FRUIT

The term pome fruit encompasses well-known crops such as apples and pears but also less-known commodities as quince, rowan, loquat, toyon and whitebeam. Pome fruits are members of the plant family *Rosaceae,* subfamily *pomoideae* (Webster and Palmer, 2017). They are generally considered low-risk commodities for foodborne illnesses. However, these commodities are very sensitive to outgrowth of *Penicillium expansum*, a mycotoxigenic mould, producing the mycotoxin patulin. Therefore, for processed apple and pear products (e.g. juice, concentrates), where often lower quality or infected fruits are used, the presence of patulin must be considered. The distinctive softness and delicate smooth surfaces of these fruits means they are easily damaged by impact or friction; hence, they are not always washed or treated with aqueous sanitizers. Application of water in post-harvest activities is mainly conducted to remove pesticides' residues or dust but also to reduce the heat (cooling), and to sort the fruits and their transportation in a packhouse. Typically, pome fruits are subjected to controlled atmosphere (i.e. ultra-low oxygen conditions) and cold storage to reduce their metabolic activity and to preserve the fruits year round.

5.4 MICROBIOLOGICAL HAZARDS IN POME FRUIT

Contamination of the surface of the (mature) fruit may increase during harvest and packing, through exposure to handlers and via cross-contamination from food contact surfaces and packaging. The extent to which pathogens may adhere, attach, colonize and survive on pome fruits is a critical issue. The surface of the skin is formed by the cuticle, an extracellular hydrophobic coating composed of a cutin polyester polymer, which consists of esterified fatty acids and cuticular waxes. The smooth surface of apples and pears is not a good adhesion surface for pathogens.

At a low-inoculation level (3 log CFU per apple), *L. monocytogenes* inoculated at the stem end and the equatorial surface survived but did not grow on fresh Gala and Granny Smith apples stored at 25 °C for 49 days (Salazar *et al.,* 2016). Although certain conditions did not support growth, the pathogen was always detectable by enrichment culture. The inoculation procedure had a significant effect on results; when the inoculum was allowed to dry for 24 h at 5 °C, growth was significantly slowed compared with inoculum allowed to dry for 2 h at 25 °C (Salazar *et al.,* 2016). Survival of *Salmonella* on apples stored at room temperature showed that the pathogen was capable of surviving for 12 days, only showing a significant drop at the end of the experiment (Perez-Rodriquez, Begum and Johannessen, 2014). As stated above, acid resistant STEC may also survive on the apples or in the juice. In relation to the intrinsic properties of the flesh of pome fruits, it is typically acidic and protective against the growth of the main foodborne pathogens such as *Salmonella* sp. and *Listeria monocytogenes*. The pH of apples is typically less than 4.0 (even reported as 3.5) (Beuchat, 2002).

The most important strategy for improving the safety of pome fruits involves hygienic handling and hygiene control including environmental monitoring during the sorting and packing of these products. Keeping the packing environment and packaging equipment free from contamination is essential by an effective cleaning and disinfection plan and preventive technical maintenance to avoid microbiological proliferation in a production site. Emphasizing that implementing preventive pathogen controls in packing houses is essential (Ruiz-Llacsahuanga *et al.,* 2021).

Good hygiene is essential for ensuring product safety at each stage of the production and processing of pome fruits. This will normally be achieved through the application of GAPs, GHPs and GMPs. The Codex *General Principles of Food Hygiene* provide general guidance for ensuring food hygiene and a foundation for further commodity-specific codes of practice and guidelines (FAO and WHO, 2021a).

5.5 MITIGATION AND INTERVENTION MEASURES EVALUATED WITH POME FRUIT

5.5.1 Post-harvest applications

5.5.1.1 Prevention of cross-contamination during washing

Unlike many forms of fresh produce, the stem scar on pome fruits is not reported in the literature as the main site of contamination by pathogens. Nevertheless, due to the delicate nature of most pome fruits, they require care during handling and prompt removal of field heat. Apples are often transported using water and held in water baths for prolonged periods. Fresh produce operations that use flume water are very effective in transferring contamination and much less effective in removing it. This, together with the fact that the water used inside the production lines is not frequently refreshed, with use up to one week or longer and not always at refrigerated conditions, can result in a microbiological "broth" of pathogens, spoilage microorganisms and mould spores.

This was demonstrated in the work of Perez-Rodriguez *et al.* (2014), in which the cross-contamination of apples by *Salmonella* during processing of commercial fresh apples and the pathogen's survival on apples at room temperature was followed. Simulated post-harvest handling at a laboratory scale in which an apple artificially contaminated with *Salmonella* at different concentration levels (8, 6 and 5 log CFU/apple) was introduced in one batch of apples and processed through a simulated transport/washing step and a drying step using sponges to mimic the porous material used in the industry. Results indicated that at 8 log CFU/apple, 50 percent uninoculated apples were contaminated post-processing, with all analysed environmental samples (water and sponges) positive for the pathogen. However, at lower inoculum levels (5–6 log CFU/apple), no cross-contamination was detected in apples, and only environmental samples showed contamination by *Salmonella* post-processing.

Washing pome fruits is mainly practiced to remove agricultural chemicals and dust residues. If water is used, it must meet the microbial standards of drinking water, involving validated treatments such as chlorination, filtration, ultraviolet light or ozonation to avoid proliferation of microorganisms in the process water and cross-contamination amongst fruits. A study by Kenney *et al.* (2001) assessed the effectiveness of washing and rubbing in physically removing *E. coli* O157:H7 on the surface of apples. The location of cells on or in undamaged and bruised areas of apples that were not washed or rubbed did not differ significantly. Washing apples resulted in an approximate 2 log reduction in CFU of *E. coli* O157:H7 per cm^2 of apple surface. On unwashed apples, cells were detected at depths up to 30 µm below the surface. No *E. coli* O157:H7 cells were detected at locations more than 6 µm below the surface of washed apples. Cells that remained on the surface of rubbed apples appeared to be sealed within naturally occurring cracks and crevices in waxy cutin platelets. These cells may be protected from disinfection and subsequently released when apples are eaten or pressed for cider production (Kenney *et al.,* 2001).

Various decontamination treatments have been proposed to reduce the levels of pathogenic microorganisms on the surface of fruits. However, the degree of reduction achieved is typically low. Plus, the cost, feasibility, impact on fruit quality and effect on shelf-life are rarely reported.

5.5.1.2 Ultraviolet light

Ultraviolet C irradiation of apple peels immersed in water resulted in a 2 to 3 log reduction of *E. coli*, *Salmonella* and *L. monocytogenes* at UV-C doses below 500 mJ/cm^2 (Nicolau-Lapeña *et al.*, 2022). Efficacy of UV-C light irradiation is also correlated with fruit surface roughness, contact angle and surface energy, as fewer hydrophobic fruits with smooth surfaces (pears or apples) are more susceptible to UV-C irradiation than hydrophobic and rough surface fruits (cantaloupe or strawberry) (Adhikari *et al.,* 2015).

5.5.1.3 Antimicrobial dips and sprays

The efficacy of washing pome fruit may be enhanced by the use of antimicrobials such as electrolyzed water, chlorine dioxide and photocatalysis. These washing treatments have been found to have a high disinfectant activity on the epiphytic microbiota of pome fruits and are effective for the control of post-harvest rots. Some studies investigated the efficacy of applying the principle of the hurdle technology to reduce microbial population of pome fruit. For instance, Pietrysiak *et al.* (2020) used a hurdle technology for whole fresh apples based on a washing step containing surfactants, lauric arginate, sodium dodecyl sulfate, and Tween®20, combined with peracetic acid (PAA), followed by hot air impingement drying. They concluded that washing apples with solutions containing surfactants combined with PAA followed by hot air impingement drying helped to reduce the microbial loads to some extent and may help to reduce drying times significantly. On the other hand, Pietrysiak *et al.* (2019) indicated that the application of the hurdle technology and rotating use of sanitizers to avoid development of bacterial biofilm resistance may give the best results, although not conclusively. Despite the microbial reductions reported by these studies, the suitability of these treatments under commercial conditions may be low.

5.5.1.4 Edible coatings or waxes

Often apples are treated with edible wax to avoid post-harvest decay and transpiration losses. The study of Macarisin *et al.* (2019) evaluated the effect of conventional fruit coating with wax on the survival of *L. monocytogenes.* After 2 months of storage, significantly ($p < 0.05$) larger *L. monocytogenes* populations were recovered from apples coated with wax than those unwaxed, regardless of the cultivar. No differences in survival amongst *L. monocytogenes* strains (serotypes 1/2a and 4b) from clinical, food, and environmental sources were observed. The novel observation was that coating with wax facilitates prolonged survival of *L. monocytogenes* on whole apples. Therefore, more research is needed to determine if edible coatings can be used as an antimicrobial treatment or if they actually favour microbial growth.

5.5.1.5 Sorting and packaging

Optical sorting is typically conducted to remove apples infected with *Penicillium expansum*. While not inherently impacting contamination, sorting and packaging may provide conditions that enhance pathogen growth by influencing microenvironments and humidity.

5.5.1.6 Storage and ultra-low oxygen storage

Fresh apples are typically stored for up to 1 year commercially, and optimal storage temperatures for preserving quality will differ depending on apple variety. There is little information available about *L. monocytogenes* survival on fresh apples under different storage temperatures. While *L. monocytogenes* did not proliferate on apple surfaces during 12 weeks of refrigerated storage, only a limited reduction of *L. monocytogenes* was observed in this study (Sheng *et al.,* 2017). Therefore, the apple industry cannot rely on cold storage alone to control this pathogen.

Additional interventions are needed to eradicate *Listeria* on fresh apples during long-term cold storage (Sheng *et al.,* 2017). Sheng *et al.* (2018) experimented with continuous low dosing of ozone in commercial cold storage of Fuji apples (air conditions and low-oxygen conditions) and concluded that ozone gas has the potential to be used as a supplemental intervention method to control *Listeria* spp. A more recent study demonstrated the control of *Listeria* over a 9-month refrigerated storage of Red Delicious apples by use of low-dose continuous gaseous ozone in the atmosphere of the storage facilities (Shen *et al.,* 2021).

The review by Guan *et al.* (2021) concluded that gaseous interventions are suitable for *L. monocytogenes* decontamination on apples. For example, cold storage of apples, which requires waterless interventions, may benefit from gaseous antimicrobials such as chlorine dioxide and ozone. To reduce the contamination risk during cold storage, research is still needed to develop effective methods to reduce microbial loads on fresh apples. This requires commercial-scale validation of gaseous interventions and integration with existing apple cold-storage practices. Additionally, the impact of the interventions on final apple quality should be taken into consideration.

5.6 OVERVIEW OF STONE FRUIT

In some stone fruits, such as a peach, the surface is covered by a dense indumentum composed of trichomes (fine hairs). This gives the peach its typical furry surface, which provides protection against environmental factors and attack by plant pathogens. Trichomes confer a non-polar character to the peach surface, and processes which result in their removal make the fruit more susceptible to interactions with water, water-soluble compounds, and contaminants.

5.7 MICROBIOLOGICAL HAZARDS IN STONE FRUIT

In a study of pathogen survival along a simulated commercial export chain, *E. coli* O157:H7 and *L. monocytogenes* were found to survive on artificially contaminated peaches and plums (Collignon and Korsten, 2010). In a situation where high inocula were applied to fruit, time and temperature regimes did not suppress pathogen populations, indicating potential food safety issues requiring intervention strategies. Specifically, a significant increase was observed following 4 °C storage for 13 to 20 days, with no significant difference between 20 and 21 days on peach surfaces (Collignon and Korsten, 2010).

The intrinsic properties of the flesh of stone fruits are typically acidic and protective against the growth of the main foodborne pathogens such as *Salmonella* spp. and *L. monocytogenes*. The pH ranges for different fruits are white nectarine (3.98–4.32), yellow nectarine (3.63–3.88), white peach (4.34–4.98), yellow peach (3.50–3.72), and plums (3.84–4.35).

The most important strategy for improving the safety of stone fruits involves hygienic handling and hygiene control including environmental monitoring during the sorting and packing of these fruits (Williamson *et al.,* 2018). Keeping the packing environment and packaging equipment free from contamination is essential. Kuttappan *et al.* (2021) found that handling conditions for stone fruit did not favour *Listeria* growth, but once the fruit was contaminated, the pathogen would survive, emphasizing that implementing preventive pathogen controls in packing houses is essential.

5.8 MITIGATION AND INTERVENTION MEASURES EVALUATED WITH STONE FRUIT

5.8.1 Post-harvest applications

5.8.1.1 Antimicrobial dips and sprays

The efficacy of washing stone fruit may be enhanced using antimicrobials such as electrolyzed water, chlorine dioxide, and photocatalysis (Calvo *et al.,* 2019). These washing treatments have been found to have a high disinfectant activity on the epiphytic microbiota of stone fruits and are effective for the control of post-harvest rots. But there are limited studies on their efficacy against pathogens on stone fruit, and there is scant evidence of industry uptake.

5.8.1.2 Edible coatings or waxes

Nanoemulsions containing lemongrass oil have been proposed as a coating for plums, with the goal of improving microbial safety and enhancing physicochemical properties during storage (Kim *et al.,* 2013). The coatings were assessed as having the potential to inhibit *Salmonella* and *E. coli* O157:H7 contamination of plums and may extend plum shelf-life, but as with chemical treatments there remain questions about practicality, cost, and impact on sensory properties.

6

Seeded and root vegetables

6.1 OVERVIEW OF SEEDED AND ROOT VEGETABLES

This category of crops is quite expansive in the commodities encompassed which include those that grow above ground such as the Solanaceae family (e.g. tomato, pepper, eggplant), Cucurbitaceae (e.g. cucumber, squash, zucchini), Brassicaceae (e.g. broccoli, cauliflower, cabbage), Fabaceae (e.g. peas, beans, lentils), as well as root vegetables spread across many plant families (e.g. onions, carrots, radishes). Therefore, interventions that work well in one crop may not necessarily translate to success in others.

6.2 MICROBIOLOGICAL HAZARDS IN SEEDED AND ROOT VEGETABLES

Within seeded and root vegetable production systems, there are many preventive measures farms can employ that are based upon GAPs, GHPs and GMPs (FAO and WHO, 2017; FAO and WHO, 2021a; FAO and WHO, 2021b). Even with strong programmes used throughout the continuum of growing, harvesting, holding and packing produce, contamination with foodborne pathogens cannot be completely avoided given that these products are vulnerable to environmental contamination during production. For this reason, it is important to explore additional mitigation strategies that could be applied to seeded and root vegetables, while understanding the importance of GAPs, GHPs and GMPs.

6.3 MITIGATION AND INTERVENTION MEASURES EVALUATED WITH SEEDED AND ROOT VEGETABLES

For decades, researchers have been evaluating interventions that can be applied to seeded and root vegetables. Many of these interventions have not been applied at the commercial scale, which is an important distinction given that there will be significant work for industry uptake. Caution should be taken for interventions that have shown promise at the lab scale because factors such as product volume, uniformity of treatment, and organic load can drastically affect the efficacy of interventions.

6.3.1 Post-harvest physical treatments

6.3.1.1 Irradiation

Ionizing irradiation is a non-thermal processing method that has been utilized through E-beam, gamma and x-ray technologies and has been used for a wide array of purposes ranging from disinfestation of insects to inactivation of microorganisms. All products must be evaluated to determine the dose required to achieve the food safety goals while maintaining product quality. Within the realm of seeded and root vegetables, a 1.6 kGy dose supplied by e-beam was determined to be sufficient for 5 log inactivation of *Salmonella* on green onions, with D-values reported from 0.26–0.32 kGy (Murugesan *et al.,* 2011). At this dose, the product did not demonstrate any differences in quality from the untreated control and was able to slightly extend shelf-life. A dose of 1 kGy supplied by gamma radiation reduced *Salmonella* populations on baby carrots and grape tomatoes by 3.7 to 5.6 log CFU, respectively (Berrios-Rodriguez *et al.,* 2022). Treatment of seeds has also been evaluated with a 7 kGy dose resulting in a 4 log reduction of *Salmonella* on tomato seeds and no impact on germination rates (Trinetta *et al.,* 2011). While this technology has been evaluated with food systems for quite some time, consumer acceptance is a major hurdle to overcome for widespread adoption in the future.

6.3.1.2 Ultraviolet light

Ultraviolet C has been used as a non-thermal surface treatment for decontamination of all types of surfaces including that of fresh vegetables. A > 3 log reduction of *Salmonella* was achieved on green tomatoes when treated with a UV dose of 22.3 mJ/cm^2 without signs of dark repair or photoreactivation post-treatment (Lim and Harrison, 2016). *Salmonella* and *E. coli* O157:H7 were reduced > 2 log CFU/tomato when treated with 60 mJ/cm^2 UV-C, with less effect when the stem scar was inoculated (1.6–1.9 log CFU) demonstrating how surface characteristics can play a role in UV efficacy (Mukhopadhyay *et al.,* 2014). Much higher doses ranging from 500–4 000 mJ/ cm^2 did not demonstrate significantly higher inactivation rates for *Salmonella* or *L. monocytogenes* when Roma tomatoes and jalapeno peppers were treated (2.59–3.82 log CFU/g) (Sommers, Sites and Musgrove, 2010). When treated in polyethylene film, which does block some UV-C light, non-pathogenic *E. coli* was reduced up to 1.6 log CFU/g with a UV dose of 746.6 mJ/cm^2 (Abdussamad, Rasco and Sablani, 2016).

6.3.2 Post-harvest aqueous treatments

Chemical treatments are the most studied interventions for reduction of microbial contamination in fresh fruits and vegetables, either through prevention of cross-contamination during processing and handling or by direct reduction of microbial load on the product. The treatments can be applied during post-harvest processing or during storage. The commodities can be treated in bulk or in individual packages. Efficacy of chemical treatment is influenced by how the treatment is applied (aqueous, gaseous, or aerosolized). Delivery of chemical treatment can be facilitated by physical means (e.g. sonication and vacuum impregnation). Combination treatments with multiple chemical treatments or combined chemical and physical methods can enhance treatment efficacy. Examples of decontamination methods that have been applied to seeded and root vegetables are summarized below. Further validation of these interventions under commercial conditions and regulatory approval will be needed before their use commercially.

6.3.2.1 Prevention of cross-contamination during washing

Washing is frequently applied during post-harvest processing of seeded and root vegetables to remove dirt, debris and other contaminants. Antimicrobial chemicals are added in the wash water to prevent spreading of microbial contamination in the production batch. Commonly used antimicrobial chemicals for produce washing include chlorine (as sodium or calcium hypochlorite), peracetic acid, chlorine dioxide, and ozone (Gombas *et al.,* 2017).

The use of sufficient sanitizers in wash water is critical to minimize the potential of cross-contamination. Many factors may influence the effectiveness of sanitizers and thus the amount needed in wash water, including concentration, pH, organic load, water temperature, product-to-water ratio, rate of water replenishment, and water agitation (USFDA, 2018). Lab-scale studies have been conducted to determine the free chlorine level needed to prevent *Salmonella* cross-contamination of tomatoes in model flume systems and how the minimum effective chlorine level may be influenced by the inoculation level, contact time and presence of organic matter (Sreedharan *et al.,* 2017; Bertoldi *et al.,* 2021).

Industry guidelines have recommended specific performance criteria to maintain the quality of post-harvest wash water, e.g. maintaining free chlorine concentrations at high levels (> 50 ppm) (International Fresh Produce Association, 2018, 2019), yet these standards have not been adequately validated. Guidelines for validating antimicrobial washes for fresh-cut leafy greens have been developed (Gombas *et al.,* 2017) which may serve as examples for validating washing processes for other fresh produce commodities.

6.3.2.2 Antimicrobials applied as dips, sprays, and through aerosolization

Chlorine-based biocides (e.g. sodium and calcium hypochlorite) are the most used sanitizers in the commercial production of fresh produce, especially during the washing step. The effectiveness of these sanitizers, at the concentration of use, varies between commodities and between published studies (Goodburn and Wallace, 2013; Gombas *et al.,* 2017; Yoon and Lee, 2018; Deng *et al.,* 2020). For tomatoes, studies showed that treatment with 200 ppm chlorine for up to 3 min achieved a reduction in *Salmonella* of 1–2 log (Beuchat, 1998; Sapers and Jones, 2006), but in other studies, a higher log reduction was observed. Sreedharan *et al.* (2017) showed that washing tomato in chlorinated water (HOCl concentration 100 ppm) containing a high-organic load prevented *Salmonella* cross-contamination in a model flume system and reduced the *Salmonella* levels by > 4.5 log CFU/tomato. Felkey *et al.* (2006) showed that washing tomatoes in a model flume system containing 150 ppm free chlorine (pH 6.5) at 25 °C for up to 2 min reduced the *Salmonella* population on the smooth surface by 6.4 log, but *Salmonella* inoculated on puncture wounds or stem scar were reduced by only 0.7 or 1.9 log units, respectively. Similar reductions in *Salmonella* counts were observed when the temperature of the wash water was raised to 35 °C. However, these extremely high chlorine concentrations (≥ 100 ppm) are no longer recommended, and maintenance of a residual free chlorine of about 25 ppm at the optimum pH (6.0) and temperature (< 4 °C) have been recommended to avoid cross-contamination but also to reduce the formation of disinfection by-products such as chlorates (Gombas *et al.,* 2017).

Chlorine production through electrolyzed water (EW) for onsite generation has been evaluated for post-harvest washing. Washing tomatoes in neutral electrolyzed water (155 ppm free chlorine, pH 6.5) for 5 min reduced *E. coli* O157:H7 and *Salmonella* Typhimurium DT 104 by 4.8 and 5.4 log CFU/tomato, compared with a reduction of 2.5 or 3.2 log CFU/tomato after washing in deionization water (DI water). Both pathogens were completely inactivated in the wash water, suggesting that neutral electrolyzed water is effective in preventing crosscontamination (Afari *et al.,* 2016). Deza, Araujo and Garrido (2003) showed that treatment of tomato with neutral EW (chlorine concentration 89 mg/L, pH 8, and 23 °C) for 30 sec reduced populations of *E. coli* O157:H7, *Salmonella* Typhimurium and *L. monocytogenes* by 4.4, 3.7 and 4.7 log CFU/cm^2, respectively, compared with a 2 log reduction of all three pathogens in tomatoes treated with DI water.

Park *et al.* (2009) used acidic EW containing 37.5 ppm of available chlorine at pH 2.06 to treat grape tomatoes inoculated with *E. coli* O157:H7, *Salmonella* Typhimurium or *L. monocytogenes* and found that all three pathogens decreased by > 5 log CFU/g after a 1 min treatment. But the efficacy of EW was much lower in the presence of added organic matter.

Large or pilot plant scale studies have been conducted to determine the efficacy of sanitizer on produce and in preventing cross-contamination. Washing with 25 ppm free chlorine resulted in cross-contamination of tomatoes when the inoculum load of *Salmonella* was 6 log CFU/tomato in water with 300 mg/l COD, but cross-contamination was prevented under these challenging conditions when the inoculum was log 4 CFU/tomato (Bertoldi *et al.,* 2022). Wang and Ryser (2014) found that washing tomatoes (~ 11.3 kg) in a pilot-scale processing line containing 890 L of EW (40 ppm of available chlorine, pH 6.7) for 2 min yielded a significantly lower log reduction for *Salmonella* on tomatoes compared with that observed in tomatoes washed in water containing 40 ppm of chlorine adjusted to pH 6.0 with citric acid (2.1 log vs 3.1 log CFU/g). The authors attributed this difference to the lower pH, means of inoculation, and/or method of exposure. Although inexpensive to produce, the relatively slow rate of generation of EW may limit its usefulness for large-scale processors.

Chlorine dioxide (ClO_2) effectiveness for decontamination of vegetables is relatively limited (Han *et al.,* 2001; Yoon and Lee, 2018). Pao *et al.* (2007) found that, while immersion of tomatoes in 5 ppm ClO_2 solution completely prevented cross-contamination by *Salmonella*, populations of the pathogen on air-dried tomatoes were not significantly reduced after immersion in ClO_2 solutions at ≤ 20 ppm for 1 min.

Aerosolization of aqueous ClO_2 resulted in a better penetration capability and an improved treatment efficacy. Cho *et al.* (2017) showed that treatment with aerosolized ClO_2 at 400 ppm for 30 min reduced *E. coli* O157:H7, *Salmonella* Typhimurium and *L. monocytogenes* on washed carrots by 2.4, 2.3 and 2.1 log, respectively. Chlorine dioxide residues were 1 ppm or less in all treated carrots, showing no appearance or discolouration defects. Jiang *et al.* (2017a, b) evaluated the efficacy of in-package treatment of aerosolized aqueous sanitizers in reducing *Salmonella* Typhimurium on cherry tomatoes. A 1-min treatment with aerosolized ClO_2 at ≥ 100 ppm achieved a > 5 log reduction of *S.* Typhimurium on the smooth surface of tomatoes. Four hundred ppm aqueous ClO_2 achieved a 4.89 log reduction in *Salmonella* population in the stem scar area. The efficacy of ClO_2 treatment increased during the 3-week storage at 10 °C, achieving a ~ 6 log inactivation of *Salmonella* in the stem scar area.

Hydrogen peroxide (H_2O_2) The antimicrobial efficiency of H_2O_2 as a wash sanitizer is generally low, being comparable to 100–200 ppm of chlorine treatment at concentrations of 4–5 percent (Ölmez and Kretzschmar, 2009). Aerosolization can enhance the penetration capability of sanitizers. There has been increasing interest in the use of aerosolized biocides for decontamination of fresh produce (Jiang *et al.,* 2017a; Song and Fan, 2020). Song and Fan (2020) showed that aerosols generated from 7.8 percent of H_2O_2 reduced the level of *Salmonella* Typhimurium on smooth surface and stem scar of grape tomatoes by 3.17 and 1.54 log CFU/tomato, respectively.

Ozone applications for post-harvest treatment and vegetables has been reviewed (Deng *et al.,* 2020; Fan, 2021; Goodburn and Wallace, 2013; Horvitz and Cantalejo, 2014; Warriner and Namvar, 2014; Yoon and Lee, 2018). Ozonated water is used for washing a variety of fresh fruits and vegetables to reduce microbial load and prevent cross-contamination (Yoon and Lee, 2018; Deng *et al.,* 2020). Washing broccoli florets in ozonated water at 7 ppm for 2.5 min and 5 min reduced inoculated *L. monocytogenes* by 1.02 and 1.15 log, respectively, compared with reductions of 0.63 and 0.89 log CFU/g observed in broccoli washed in sterile water (Severino *et al.,* 2014). Alexandre *et al.* (2011) showed that washing red pepper in ozonated water at 2 ppm for 3 min added an additional 0.5 to 1.0 log reduction in *L. innocua* inoculated on pepper compared with that found on pepper washed in water alone. Tomatoes washed in ozonated water at 0.5 mg/L for 15–30 min reduced *E. coli* by 2.0–2.9 log CFU/fruit (Venta *et al.,* 2010). Excessive exposure to ozone can result in discolouration, loss of flavour/aroma, and degradation of phytochemicals (Yoon and Lee, 2018).

Organic acids have been evaluated for reduction of pathogens on fresh vegetables (Deng *et al.,* 2020; Yoon and Lee, 2018). Their efficacy varies among published studies, depending on acid type, concentration, treatment time, microorganism and commodity. Peroxyacetic acid (PAA) is an organic acid that has been widely used in the produce industry and is approved for applications on fruits and vegetables (up to 80 ppm in wash water). Unlike that of chlorine-based sanitizers, the efficacy of PAA is only minimally impacted by changes in pH and organic load of the wash water (Wang and Ryser, 2014). Treatment with PAA (75 ppm) for 60 or 120 s in the simulated flume reduced *Salmonella* populations on smooth surfaces of bell peppers and cucumbers to below detection (about a 4 and 5 log reduction, respectively). The same treatment reduced *Salmonella* populations in stem scar of the two commodities by 2.5–3 log CFU/g and that inoculated on puncture wounds by < 2 log CFU/g (Yuk *et al.*, 2006). Singh *et al.* (2018) washed tomato in an automated washer with 85 ppm PAA reduced *Salmonella* and *E. coli* O157:H7 by 6.8 and 5.4 log CFU/g. Wang and Ryser (2014) assessed the ability of 40 ppm of peroxyacetic acid to reduce *Salmonella* on tomatoes, in wash water, and on equipment surfaces using a pilot-scale processing line. They showed that washing tomatoes in PAA for 2 min reduced *Salmonella* on tomatoes by 2.5 log CFU/g and reduced *Salmonella* in wash water by > 5 log CFU/ml. The efficacy of PAA applied in an overhead spray and brush roller system was examined for reducing *Salmonella* on tomatoes. A 60-s spray treatment with 80 ppm of PAA decreased *Salmonella* populations by > 5 log on tomato surface, similar to that found when 100 ppm sodium hypochlorite was applied (Chang *et al.*, 2012). However, it should be noted that the efficacy of PAA in industrial settings has not been able to be demonstrated as much as that of chlorine (López Gálvez *et al.*, 2020).

The efficacy of other organic acids used as antimicrobial wash has been investigated. Velázquez *et al.* (2009) found that treatment with 0.2 percent and 1 percent lactic acid for 1 min reduced *E. coli* O157:H7 on tomatoes by 2.2 log CFU/tomato. Singh *et al.* (2018) found that washing with 2 percent lactic acid for 5 min in chilled water (4 °C) reduced *Salmonella* and *E. coli* O157:H7 by 4.8 and 2.4 log CFU/g. Treatment with citric acid for up to 3 percent for 15 min did not lead to a significant reduction in *E. coli* inoculated on carrots and can only achieve a low inactivation (0.7 log) of *E. coli* inoculated on tomatoes (Bermúdez-Aguirre and Barbosa-Cánovas, 2013; Barbosa-Cánovas *et al.*, 2013). Gurtler *et al.* (2012) showed that relatively high concentrations of combined organic acids were effective at reducing *Salmonella* inoculated onto the stem scar of red round tomatoes during 2-minute immersion treatments. Treatment with 2 percent and 6 percent total of lactic+ acetic acid reduced *Salmonella* by 4.4 and 5.5 log CFU/stem scar. Treatment with 1, 3 and 6 percent total of lactic+acetic+levulinic acids reduced *Salmonella* by 2.2, 4.4 and 6.9 log, respectively, compared with a reduction of 1.91 log CFU/stem scar when treated with 90 ppm of PAA. The antimicrobial efficacy of organic acids may be enhanced with the combined use of surfactants. Washing grape tomatoes in citric or lactic acid (0.35 to 0.61 percent) with two surfactants generally recognized as safe (0.025 percent sodium-2-ethyl-hexyl sulfate and 0.025 percent sodium dodecylbenzene-sulfonate) for 2 min reduced *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* on grape tomatoes by up to 4.90, 4.37 and 3.98 log CFU/g, respectively (Gurtler, 2020).

Aerosolization of organic acids for in-package decontamination of fresh produce has been investigated. Jiang *et al.* (2017a) evaluated the efficacy of aerosolized aqueous sanitizers in reducing populations of *Salmonella* Typhimurium on cherry tomato packaged in clam-shells. Treatment with 400 ppm PAA, or two organic acid mixtures (2 percent lactic acid + 2 percent acetic acid + 2 percent levulinic acid or 3 percent acetic acid + 3 percent lactic acid) for 1 min reduced populations of *S.* Typhimurium on smooth surfaces > 5 log CFU/fruit and that on the stem scar by 2.6, 1.9, or 1.7 logs, respectively. During 3 weeks of storage at 10 °C, *Salmonella* populations on fruit treated with the acid combinations were reduced by additional 1.4–1.6 log.

Incorporation of vacuum impregnation in a produce washing process helps to improve the delivery of sanitizers to protected sites and thus enhance antimicrobial efficacy of the wash water. Kang and Kang (2017) showed that vacuum impregnation (21.3 kPa) applied during washing in 2 percent malic acid reduced levels of *Salmonella* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes* on paprika fruit and carrot from 5–7 log CFU/cm^2 to below detection (< 1 log CFU/cm^2) after 3–5 min and 15–20 min, respectively. The colour, texture and titratable acidity values of treated paprika and carrots were not significantly different from those of untreated control samples during the 7-day storage.

Natural antimicrobials. Essential oils have been evaluated as vapour phase treatments and as post-harvest washes with and without emulsification, with the vast majority of work evaluated in tomatoes in lab-scale experiments. Typical concentrations required for antimicrobial efficacy are in the range of 0.5 to 2 percent (v/v) for whole oil (e.g. thyme oil, clove bud oil) or 0.2 to 1 percent (v/v) when applying purified active compounds (e.g. thymol, eugenol, carvacrol) (Dunn *et al.,* 2019; Gündüz, Gönül and Karapınar 2010b; Landry *et al.,* 2016; Lu and Wu, 2010; Mattson *et al.,* 2011; Yun, Fan and Li, 2013). Thymol (0.4 percent) or thyme oil (2 percent) rinses of *Salmonella*-inoculated cherry tomatoes resulted in a > 4 log reduction after 5 min and were reported not to cause any organoleptic changes to the finished product (Lu and Wu, 2010). Similar wash interventions have also been evaluated with eugenol, cinnamaldehyde and carvacrol on plum tomatoes which resulted in a > 6 log reduction of *Salmonella* at the highest concentrations evaluated (0.75 percent; Mattson *et al.,* 2011).

When used in the vapour phase, mustard essential oils and allyl isothiocyanante caused a > 5 log reduction of *Salmonella* but also resulted in negative organoleptic changes ranging from decreased firmness to ascorbic acid and lycopene decreases (Yun, Fan and Li, 2013). Cinnamon essential oil, carvacrol and cinnamaldehyde resulted in a > 3 log reduction without negative changes to the treated tomatoes. Carvacrol, cinnamon essential oil and cinnamaldehyde achieved 3.37, 4.56 and

3.79 log CFU/g reductions of *S.* Typhimurium, respectively, and did not affect the colour, texture, level of ascorbic acid, or lycopene content.

6.3.3 Post-harvest gaseous treatments

6.3.3.1 Gaseous treatment with chlorine dioxide

The gaseous form of ClO_2 has a greater penetration capacity and has been shown to be more effective than aqueous ClO_2 in reducing microbial contamination. Han *et al.* (2000) showed that treatment with 3 ppm gaseous ClO_2 for 10 min at 20 °C reduced *L. monocytogenes* population on green pepper by 7.4 or 3.7 log units on uninjured and injured surfaces, respectively, compared with 3.7 and 0.4 log reductions achieved by treatment with 3 ppm aqueous ClO_2. Gaseous ClO_2 was also effective in reducing *E. coli* O157:H7 population on green pepper, achieving a 6.5 log reduction after a 30 min treatment with 1.2 ppm gaseous ClO_2 (Han *et al.,* 2000). Lee *et al.* (2018) showed that chili peppers treated with ClO_2 gas (peak concentration 357 ppm) for 6 h at 25 °C and 100 percent relative humidity reduced *S.* Typhimurium (initial level 5.6 log CFU/g) population to below detection (< 1 CFU/10.8 g) without affecting the colour or moisture content of the treated chili peppers.

Sy *et al.* (2005) reported that treatment of tomato and onion with gaseous ClO_2 at 4.1 ppm for 25 min reduced *Salmonella* by 4.33 log CFU/tomato and 1.94 log CFU/onion, respectively, without markedly adverse effects on sensory qualities. Bhagat, Mahmoud and Linton (2010) showed that treatment of tomatoes with 0.5 mg/L ClO_2 gas for 12 min at 22 °C and a relative humidity of 90 percent reduced *Salmonella* and *Listeria* by > 5 log units on the tomato skin and extended the shelf-life by 7 days. The efficacy of ClO_2 gas was significantly influenced by ClO_2 level, exposure time and treatment temperature when applied to grape tomatoes. Grape tomatoes exposed to 0.15–0.85 mg of ClO_2 gas for up to 58 min resulted in population reductions of *S.* Typhimurium of up to 3.95 and 7.3 log CFU/fruit, when treatments were conducted at 4 and 25 °C, respectively (Netramai *et al.,* 2016).

Variations in relative humidity greatly influence the solubilization of ClO_2 gas on tomato surfaces which is positively correlated with the level of inactivation of pathogens (Park, Kim and Kang, 2018). Exposure to 30 ppm of ClO_2 gas (50 percent relative humidity) for 20 min resulted in ~ one log reduction of *E. coli* O157:H7, *Salmonella* Typhimurium and *L. monocytogenes* on tomato surfaces. When the tomatoes were treated with 30 ppm ClO_2 gas at 90 percent relative humidity, populations of the three pathogens reduced to below detection (< 0.48 log CFU/cm^2) within 10 min of exposure (Park, Kim and Kang, 2018).

The efficacy of ClO_2 gas treatment varies between commodities. It has been observed that a higher level of microbial reduction (≥ 5 log) after treatment with ClO_2 on fruits and vegetables that have smooth surfaces (e.g. tomatoes) occurs. In contrast, pathogenic bacteria inoculated on produce that have relatively rough surfaces (e.g. cucumber) showed lower reductions (Yoon and Lee, 2018). However, exceptions to this observation exist. Yuk *et al.* (2006) showed that ClO_2 gas treatment of cucumber reduced *Salmonella* cells to undetectable levels at all inoculation locations. For bell peppers, ClO_2 gas treatment resulted in approximately two log reductions for all inoculation sites. The authors attributed this inconsistency to differences in experimental conditions.

Gaseous ClO_2 treatment can also be applied during storage of fresh fruits and vegetables. A 2 kg pilot-scale study simulating industrial storage conditions was conducted to evaluate the efficacy of gaseous ClO_2 against bacterial pathogens on produce (Bridges, Rane and Wu, 2018). Five hours of ClO_2 exposure at concentrations of 0.07 mg ClO_2 per g of sample resulted in reductions of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* on tomato by > 7 log to below detection. The same treatment lowered the population of the three pathogens on baby-cut carrots by 7.7, 4.8 and 2.5 log, respectively.

A controlled-release ClO_2 pouch that was made by sealing ClO_2 into semipermeable polymer film and affixed to the inside of a perforated clamshell reduced *E. coli* populations by 3.08 log CFU/g on grape tomatoes after 14 days of storage. The ClO_2 concentration in the clam-shell reached 3.5 ppm and remained constant until day 10 and decreased to 2 ppm by day 14 (Sun *et al.,* 2017). The treatment also reduced softening and weight loss and extended the overall shelf-life of the tomatoes. Other formulations involving the use of diatomaceous earth for sustained release of chlorine dioxide gas against foodborne pathogens on produce have also been developed (Park, Kim and Kang, 2021)

6.3.3.2 Gaseous treatment with ozone

Treatment of produce with ozone in its gaseous form has a wider application. It can be applied either at the processing stage or during storage (Fan, 2021). However, gaseous sanitizers have some disadvantages, including inconsistent results and the requirement of on-site generation (Fan, Sokorai and Gurtler, 2020). The efficacy of gaseous ozone for decontamination of fresh produce varies between studies, depending on treatment methods, concentration and time, target microorganism, type of produce, inoculation and enumeration methods. Han *et al.* (2001) reported that a > 5 log reduction of *E. coli* O157:H7 on green peppers was achieved after treatment with 7 mg/L ozone for 20 and 40 min at 22 °C. Alwi and Ali (2014) showed that treatment with 9 ppm of gaseous ozone for 6 h reduced

E. coli O157:H7, *S.* Typhimurium and *L. monocytogenes* populations on fresh-cut bell pepper by 2.89, 2.56 and 3.06 log CFU/g.

Treatment with 856 mg/m^3 gaseous ozone (or 1.71 µg O_3/g of produce) reduced *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* on tomatoes and baby-cut carrots by 1.1–1.6 log and 0.5 –1.2 log CFU/g, respectively, but resulted in noticeable bleaching of carrot and tomato tissue (Bridges, Rane and Wu, 2018). Wang *et al.* (2019) showed that 6.85 mg/L ozone for 2 and 4 h treatments reduced *Salmonella* populations by approximately 2 log CFU/fruit on both the smooth surface and stem scar area of tomatoes but caused deterioration in the quality of grape tomatoes. Daş, Gürakan and Bayındırlı (2006) found that 20 ppm gaseous O_3 treatment (20 ppm) completely reduced *Salmonella* on tomato surfaces to below detection (> 7 log CFU/tomato) after 15 min. However, a surface colour change from red to yellow was observed on ozone-treated tomatoes.

A novel in-package ozonation device, capable of generating 1 000 ppm of O_3 inside sealed film bags was evaluated for its efficacy for decontamination of tomatoes and for its effect on fruit quality (Fan *et al.*, 2012). Within 40 sec of treatment, *L. innocua* on the tomato surface or stem scar was reduced to below detection or by 4 log CFU/tomato, respectively. Levels of *Salmonella* and *E. coli* O157:H7, either surface or stem scar-inoculated, were reduced by 2–3 log CFU/tomato after 2- to 3-min treatment. No negative effects on fruit colour or texture were observed during a 22-day post-treatment storage of treated tomatoes.

6.3.4 Post-harvest plasma treatments and combinations

Plasma is the fourth state of matter that creates highly oxidative species, such as atomic oxygen and ozone. Plasma is a non-thermal technology that has been applied directly to produce the water used to wash and transport produce post-harvest in lab-scale experiments. Limited efficacy (< 1 log inactivation of *Salmonella* on grape tomatoes) was observed when tomatoes were treated in containers, but there was an improved efficacy (> 3 log reduction) when the treatment was applied to rolling tomatoes (Min *et al.*, 2018). Treatment conditions did not result in differences for colour, firmness, or weight loss. When tomatoes were treated for 5–15 min, a 1–3 log reduction of *Salmonella* and surrogate *E. coli* was observed (Bermúdez-Aguirre and Barbosa-Cánovas, 2013; Prasad *et al.*, 2017; Timmons *et al.*, 2018). When incorporated into post-harvest water, a contact time of 1–2 min was required to have a greater microbial reduction on grape tomatoes compared to the chlorine controls, with additional exposure time resulting in increased inactivation of *Salmonella*, *L. monocytogenes* and *E. coli* (Hou *et al.*, 2021).

Cold plasma activation of aeros O_2 resulted in the formation of ionized hydrogen peroxide (iHP). Application of iHP further improved efficacy of H_2O_2 treatment, reducing the *Salmonella* population on the smooth surface and stem scar by 5.28 and 2.35 log CFU/tomato, respectively. The efficacy of iHP was evaluated in a pilot-scale study (Song, Annous and Fan, 2020). Although *Salmonella* Typhimurium and *Listeria innocua* inoculated on the smooth surface of tomatoes were reduced to below detection, only one log reduction was achieved against bacteria inoculated on stem scar. Treatment with iHP did not significantly affect the quality attributes of the produce.

6.3.5 Post-harvest biocontrol treatments and combinations

Bacteriocins have shown promise for inactivating foodborne pathogens on fresh vegetables, with nisin being most frequently studied in lab-based experiments. Nisin coatings (10^3 IU/ml) and washes had a > 2 log difference from untreated controls. Nisin in combination with essential oil coatings (carvacrol and mountain savoury) and irradiation (0.5 and 1 kGy) were more effective than individual treatments. Coatings with nisin and 1 kGy γ-irradiation treatment resulted in a > 6.5 log CFU/g inactivation of *Salmonella* on mini carrots within 3 days after treatment (Ndoti-Nembe *et al.,* 2015). In another study, a combination of pulsed light with a novel wash containing nisin resulted in a > 5 log reduction of *Salmonella* on tomato stem scars with the only organoleptic change being slightly softer texture at the end of shelf-life (Leng *et al.,* 2020). Another study evaluated a novel nisin-containing antimicrobial wash on cherry tomatoes, resulting in a > 2 log CFU/g inactivation of *Salmonella* outperforming 200 ppm free chlorine (Berrios-Rodriguez *et al.,* 2022).

Bacteriophage combinations have been evaluated for Gram-negative targets on fresh produce, most demonstrating some efficacy compared to control samples. Phage-treated cherry tomatoes resulted in a > 4 log difference of *S.* Newport compared to untreated controls when the multiplicity of infection (MOI) was 10^5, and two log difference was observed when the MOI was decreased to 10^3 (El-Dougdoug *et al.,* 2019). Bacteriophage have also shown activity against *Shigella* on treated cherry tomatoes, resulting in a > 3.5 log difference between control and treated samples with an MOI of 10^4 (Shahin *et al.,* 2021). Another study found an initial 2 log difference of *S.* Newport in control and phage-treated whole cucumbers when the MOI was 10^4. However, populations continued to decline on control samples when held at 10 °C and 22 °C to a point where there were no differences in *S.* Newport populations in control and treated cucumbers by day 7 (Sharma *et al.,* 2017). Coatings have also been explored for their potential to deliver phage to produce surfaces, with one study finding a > 2 log difference in

E. coli O157:H7 on tomato surfaces for chitosan films with phage incorporated (Amarillas *et al.,* 2018). The phage cocktail was stable over the course of a week, and the film did not deter phage activity (Amarillas *et al.,* 2018).

6.3.6 Post-harvest combined treatments

Combination treatments incorporating chemical and physical methods for synergetic effects in decontamination of fresh fruits and vegetables have been increasingly investigated (Yoon and Lee, 2018; Fan and Wang, 2022). The effectiveness of combining aerosolized H_2O_2 and gaseous O_3 to inactivate *Salmonella* on tomatoes has been investigated (Fan, Sokorai and Gurtler, 2020). The combined treatment is based on the principle that reaction of ozone with H_2O_2 can produce hydroxyl radicals, which are among the strongest oxidants, and that aerosolized sanitizers have a greater ability to penetrate into small crevices and protective sites where microorganisms are located. It was found that combination treatments reduced the populations by up to 5.2 log CFU/fruit on smooth surface and 4.2 log CFU/fruit on the stem scar, compared with a reduction of < 0.6 log CFU/fruit on both the smooth surface and the stem scar area, and aerosolized hydrogen peroxide alone reduced the populations by up to 2.1 log CFU/fruit on the smooth surface and 0.8 log CFU/fruit on the stem scar area.

The application of UV-C light for improved efficacy of antimicrobial washes has been investigated. Mukhopadhyay *et al.* (2015) showed that integrated treatment using a low (0.6 kJ/m^2) dose UV-C light followed by immersion in selected sanitizers (1 percent lactic acid, 1 percent citric acid or their binary mixtures, 3 percent H_2O_2 or a novel antimicrobial preparation containing hydrogen peroxide, EDTA and nisin) for 2 min achieved higher log reductions (> 4 log) in *Salmonella* populations on tomatoes compared with that obtained from treatment with combined UV-C and 200 ppm chlorine (3.95 log reduction) or 200 ppm chorine alone (2.4 log reduction).

The use of ultrasound in combination with sanitizers for enhanced antimicrobial efficacy has been investigated. Combined treatment of ultrasound at 45 kHz frequency and 40 mg/L peracetic acid (PAA) for 10 min resulted in a reduction of *Salmonella* Typhimurium on cherry tomatoes by 3.9 log CFU/g, compared with a reduction of 2.7 log CFU/g after treatment with 40 ppm PAA only (Brilhante São José and Vanetti, 2012). Combining electrolyzed water (EW) treatment with ultrasonication resulted in improved efficacy. Neutral EW combined with 210 W ultrasonication for 5 min completely inactivated *E. coli* O157:H7 and *Salmonella* Typhimurium DT 104 on tomatoes, resulting in a reduction of > 8.44 and 8.47 log CFU/tomato, respectively (Afari *et al.,* 2016).

7

Conclusions

Several technologies have been identified which show efficacy in reducing foodborne pathogens on the commodities evaluated. While there has been a great deal of work done with these commodities, it has primarily been associated with a limited number of bacterial foodborne pathogens, leaving gaps in knowledge connected to protozoan or viral targets. Additionally, among lab-scale technologies, there is a significant amount of evaluation that is necessary to discern how well these technologies work when scaled up and what impact, positive or negative, they may have with regard to shelf-life and quality.

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Annexes 1

Questions from Codex Committee on Food Hygiene

A1. Part 1

The following questions (Q1–Q7) were presented by the Codex Committee on Food Hygiene (CCFH) electronic working group (eWG) on the development of "Guidelines for the control of STEC in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts" in July 2021. The answers were addressed separately by the experts convened as part of the Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) on the Prevention and Control of Microbiological Hazards in Fresh Fruits and Vegetables.

Q1. Most control measures in Annex 2 "Fresh leafy vegetables" of the draft "Guidelines for the control of STEC in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts" are not specific for STEC (and thus information in the *Code of Hygienic Practice for Fresh Fruits and Vegetables* would suffice). JEMRA – Please provide input on control measures that have been studied scientifically with respect to control of STEC and thus warrant inclusion. (These measures may also control other pathogens, but we need to know if there is sufficient scientific information related to control of STEC to warrant including them in this annex.)

A1. Many potential measures have been scientifically studied with respect to control of microbiological hazards in fresh fruits and vegetables, including leafy greens. However, based on the experts' opinions, while much of this research was not carried out with STEC, the conclusions are valid for STEC control as well. Specific experiments using different STEC are not necessary; there is no evidence to indicate that STEC behaves differently in response to these control measures. The most significant control measures include:

- maintenance of the cold chain at every stage along the farm-to-fork continuum;
- addition of biocides to process water to prevent cross-contamination. It is noted that biocides use can reduce microbiological load on product, but the data are not sufficient to provide consistent outcomes; however, inclusion is prudent to prevent cross-contamination;
- avoiding direct application of untreated animal manures (e.g. ruminant species, pigs, poultry) to leafy vegetable fields as it may increase the likelihood of STEC contamination. Composting reduces risk of contamination, but the quality and effectiveness of composting can be variable, so the primary recommendation is to avoid the application of untreated raw manures to leafy vegetable fields in the year of production; and
- ensuring water that contacts the crop directly is fit-for-purpose. If growers

do not have the resources to monitor or determine water quality, adopting practices that prevent direct water contact with the edible part of the crop are recommended.

Q2. It has been suggested that the guidelines address HACCP system principles. Please provide input on whether good hygiene practices (GHPs) or good agricultural practices (GAPs) at a step provides adequate control of STEC or whether there are applicable critical control points (CCPs).

A2. Good hygiene practices or good agricultural practices provide an effective means of establishing farming practices, which minimize potential contamination by microbiological hazards, including STEC. Providing guidance to producers on minimizing contamination should be encouraged. For example, the introduction of HACCP system prerequisite programmes in fruit and vegetable production will reduce contamination as they include practices captured in GHPs and GAPs. It is appropriate to use the HACCP system during minimal processing activities; however, there are no CCPs that eliminate microbiological hazards.

Q3. It has been proposed that we add here that growers should be looking at distances between fields and nearby animal operations, and should be considering a minimal distance, if possible, based on recent scientific studies and publications. Is there scientific evidence to support recommendations for distance between fields growing leafy vegetables and animal operations? If not, is there specific guidance you can provide on what to consider in evaluating and controlling the risk from animal operations close to leafy vegetable growing fields?

A3. There is insufficient data to determine a minimum distance between fields and nearby animal operations, though it is noted that risks should decrease as distance increases. It is important for each operation to make an assessment based on their situation. Factors that should be considered include wildlife (e.g. type, abundance, movement), air movement and prevailing winds, hydrologic system and likely run-off, topography, human factors including intrusion and movement, and other related conditions.

Evidence indicates that the risk of airborne transport of *E. coli* O157:H7 from cattle production increases when cattle pens are very dry and when this situation is combined with cattle management or cattle behaviours that generate airborne dust (Berry *et al.*, 2015). Based on these results, distances between fields and nearby animal operations greater than 180 m would be recommended because *E. coli* O157:H7-positive leafy greens were found at that distance. However, additional research is needed to determine safe set-back distances between cattle feedlots and crop production.

Q4. Should we indicate that fresh leafy vegetables should not be harvested in areas where animal faeces are found and evaluate the risk when other evidence of animal intrusion is found? If so, what is the size of the area (e.g. around/right next to where faeces were observed? Or larger areas/field?). Is it practical to delineate an area that should not be harvested? What is the scope of vegetables which should not be harvested (e.g. Would this be limited to vegetables which are damaged by wild animals and/or contaminated by wild animal faeces)?

A4. Fresh leafy vegetables that have direct faecal contamination (visible) on the edible portion of the crop must not be harvested. There is insufficient data to provide a standard no-harvest buffer zone recommendation, but there are several considerations that should be taken into account when considering a no-harvest buffer zone. Where there is animal intrusion and evidence of localized faecal contamination, an assessment of the extent of contamination should be conducted. Factors that should be considered in determining the size of the no-harvest zones should include the extent (e.g. volume/mass/area) of contamination, the distribution of contamination (e.g. localized, widespread), type of harvest (e.g. hand, mechanical), impact of irrigation or rain influencing splash or spread and the perceived timing of the contamination (e.g. recent, past). The purpose of establishing a buffer zone is to minimize risks of direct faecal contamination as well as preventing cross contamination with equipment, hands, and harvest tools.

Q5. Can JEMRA provide advice on the role of testing of water to control STEC in fresh leafy vegetables? Is testing for STEC warranted and under what circumstances? What results would indicate a concern? Are there appropriate indicator organisms that could be used in lieu of or in addition to testing for STEC? What would be acceptable levels (or levels of concern)? What should the frequency of water testing be?

A5. The Secretariat of the Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA) does not recommend the routine testing of irrigation water for the presence of STEC. Information on testing and indicator organisms was addressed during a JEMRA meeting on the use and reuse of water in vegetable production (FAO and WHO, 2021b).

Q6. It has been suggested that we include a recommendation for storage under 7 °C here. JEMRA, does the science support this as an appropriate temperature for preventing growth of STEC in fresh leafy vegetables? Are there other temperatures combined with time that could apply?

A6. There is no convincing scientific evidence that *E. coli* O157:H7 can grow on leafy vegetables at temperatures lower than 7 °C. Moreover, there is little data available concerning the growth of non-O157 STEC in leafy vegetables. The following references are offered in support of this assessment:

Q7. The EWG is considering these alternative sentences. What is the role of testing fresh leafy vegetables for STEC and/or indicator organism (including acceptable levels of organisms or levels of concern and frequency of testing)? (See Q5 where we asked about testing water for questions that also apply to product testing.)

The working group is also considering these alternative sentences:

Microbiological testing of fresh leafy vegetables and of water for primary production for STEC is currently of limited use due to difficulty in detecting STEC resulting from low prevalence and low numbers of STEC in fresh leafy vegetables and in water.

STEC, if present, is usually only present in low numbers in fresh leafy vegetables, and this makes direct testing for these pathogens technically challenging.

Question: What is the role of testing fresh leafy vegetables for STEC and/or indicator organisms (including acceptable levels of organisms or levels of concern and frequency of testing)?

A7. Routine STEC testing at any stage is not recommended by the experts because the information derived from testing does not provide an accurate estimate of risk. It is strongly suspected that most contamination is sporadic and is non-homogeneously distributed within a lot and with low or very low numbers of contaminating microorganisms. This combination results in statistical challenges, resulting in most lots testing negative regardless of the contamination status of the lot. There are situations where targeted testing for STEC may be valuable, for example, to test system or product integrity where gross contamination is suspected. Product testing for indicators is also not recommended; however, like STEC testing, it can be useful in limited and specific situations where there is a need to verify or test a system.

A2. Part 2

The following questions (Q8–Q12) were presented by the CCFH eWG on the development of “Guidelines for the control of STEC in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts” in October 2022.

Q8. What (if any) interventions/practices/control measures have been shown to reduce STEC (or the risk of illness from STEC) during primary production of fresh leafy vegetables and post-harvest?

A8. None of the interventions/practices/control measures examined by the expert committee were deemed sufficiently effective, practical or cost effective to ensure consistent reduction in STEC on leafy vegetables either during production or after harvest.

Q9. Does the JEMRA report support the following statement? “Once product is contaminated with STEC it is not possible to eliminate it, and there are limited control measures that can be implemented to reduce it.”

A9. Yes. However, irradiation is an effective and reliable treatment for the elimination of microbiological hazards such as STEC in leafy vegetables. It is acknowledged that a range of cultural, economic, and market factors continue to impede commercial application of food irradiation. It should also be noted that some traditional preparation methods or culinary styles involve blanching or cooking of some leafy vegetable commodities before consumption, which would eliminate STEC. Additional control measures currently available such as leafy green washing using commercial sanitizers or natural extracts only manage to reduce levels of pathogens by about 1 log and sometimes even less.

Q10. Should water used for irrigation and the application of fertilizers and pesticides for fresh leafy vegetables be tested for indicator microorganisms? For STEC? If so, should microbiological testing of water be routine, periodic, or “where necessary”? If “where necessary,” what conditions would warrant such testing?

A10. It is generally agreed that methods based on the presence of indicator microorganisms do not provide a good correlation of risk associated with STEC. Testing for STEC is preferable, although technical issues (sample size, methodological complexities) may affect the accuracy, sensitivity and practicality of available analytical methods in different laboratories.

However, indicator microorganisms such as *E. coli* have been widely used to indicate the presence of faecal contamination. In general, correlation between faecal indicator organisms and pathogens is usually observed in heavily polluted waters, but this correlation becomes erratic and biologically improbable in water with a low contamination. The use of logistic regression analysis and longitudinal studies have shown high *E. coli* concentrations could reasonably predict the probability of pathogen presence (e.g. STEC) in surface water. The use of indicator microorganisms should thus be used with the correct interpretation.

The following accounts are from experts with country-specific knowledge of industry practices and some of their thoughts on water testing and risk assessment.

Spain: As in many other countries there are huge differences between growers from big and small companies. Big companies all export their products, and they have to comply with all the Private Standards that are in place (Global GAP., BRC, IFS, etc.). In these cases, they comply with the requirements included in the standards. Small growers sometimes do not even analyse the water at all, but rather do something in between, such as analysing water near harvest sights. In my opinion, even if we can have a risk assessment approach, some numbers are still needed, so I believe periodic water analyses could help the grower to validate that the assessment is somehow controlling the quality of the water.

Norway: In Norway, the requirements for water testing are rather general. According to the Norwegian Quality System in Agriculture, all water sources used for irrigation should be (risk) assessed and protected against pollution. It is required that at least one water sample per water intake is tested every year, and the result must be ready by the time the product is harvested. I think that the samples are analysed for *E. coli* and some of the wholesale companies require testing for *Salmonella* as well. I do not think it is tested for STEC. It is required that the last day of irrigation prior to harvest is documented. If the growers are certified according to the private standards, they do have to comply with the requirements there.

Africa: In Africa, commercial growers exporting their products will do annual water testing based on indicator organisms according to local legislation, Global GAP or other requirements.

Q11. What is the role of testing fresh leafy vegetables for (1) indicator microorganisms and (2) STEC?

A11. See comment above regarding the limited value of "indicator microorganisms" in the assessment of risk associated with STEC.

Regarding testing for STEC, there is a need to improve sampling protocols and analytical methods for the analysis of leafy vegetables. The expert committee thought there is no sufficient evidence to support testing before or after harvest.

Q12. Are there practices which, although they have not been shown to specifically reduce STEC, could be expected to reduce the risk from STEC, and thus should be included in the annex for fresh leafy vegetables?

A12. The expert committee are not aware of any practices that could specifically and reliably reduce STEC in leafy vegetables. Some interesting or promising approaches were noted in the scientific literature, notably the use of bacteriophage that targets STEC, but all remain at the research stage.

A3. Reference

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In 2019, following a request from the Codex Committee on Food Hygiene (CCFH), the Codex Alimentarius Committee (CAC) approved new work at its 42nd Session on the development of guidelines for the control of Shiga toxin-producing *Escherichia coli* (STEC) in leafy vegetables and in sprouts.

The objective of the report was to evaluate commodity-specific interventions used at all stages of fresh fruit and vegetable production from primary production to post-harvest activities, transportation, point of sale and consumer use. Emphasis was placed on the identification and evaluation of interventions used throughout the world to reduce microbiological hazards of fresh fruits and vegetables that contribute to the risk of foodborne illnesses, taking into consideration their effectiveness, practicality and suitability.

The expert committee addressed four subdivided commodity groups: 1) leafy vegetables and herbs, 2) berries and tropical fruits, 3) melons and tree fruits, and 4) seeded and root vegetables.

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