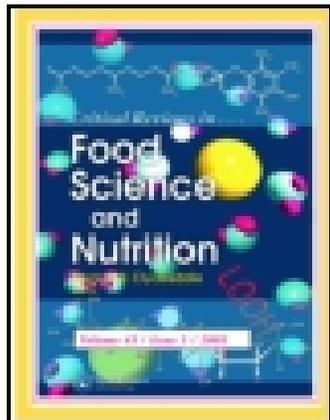


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Pre- and Post-harvest Preventive Measures and Intervention Strategies to Control Microbial Food Safety Hazards of Fresh Leafy Vegetables

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ABSTRACT

This review includes an overview of the most important preventive measures along the farm to fork chain to prevent microbial contamination of leafy greens. It also includes the technological and managerial interventions related to primary production, postharvest handling, processing practices, distribution and consumer handling to eliminate pathogens in leafy greens. When the microbiological risk is already present, preventive measures to limit actual contamination events or pathogen survival are considered intervention strategies. In codes of practice the focus is mainly put on explaining preventive measures. However, it is also important to establish more focused intervention strategies. This review is centred mainly on leafy vegetables as the commodity identified as the highest priority in terms of fresh produce microbial safety from a global perspective. There is no unique preventive measure or intervention strategy that could be applied at one point of the food chain. We should encourage growers of leafy greens to establish procedures based on the HACCP principles at the level of primary production. The traceability of leafy vegetables along the chain is an essential element in ensuring food safety. Thus, in dealing with the food safety issues associated with fresh produce it is clear that a multidisciplinary farm to fork strategy is required.

Keywords: Outbreaks, primary production, postharvest handling, processing practices, consumer handling, washing and sanitizing

INTRODUCTION

Fresh leafy vegetables, including fresh herbs, are an important part of a healthy diet (Baranowski, 2011). The increasing consumption of these types of products has increased the global demand and the need for a year round availability to consumers (Pollack, 2001). Fresh leafy vegetables are crops cultivated in various regions of the world, grown using various agricultural practices, and under different climatic conditions to fulfil the demand both of domestic and export markets (FAO/WHO, 2008a). Leafy vegetables are regularly colonized by diverse microbiota and can become contaminated with human pathogens and parasites whilst growing in the field or during harvesting, postharvest handling, processing and distribution (Beuchat, 1996; Matthews, 2009). Currently, to meet consumer demand efficiently, there is a tendency of growing leafy vegetables in large land parcels or extensive glasshouse ranges, which might increase the vulnerability of the supply chain and the potential impact of a contamination event with human pathogens. To minimize the risk associated with microbial hazards of leafy vegetables, producers and processors have at their disposal several detailed schemes or codes of practice and regulations. The associated standards and audit checklists compile the guidelines upon which the design and implementation of prerequisite chemical, physical, and biological risk reduction programs are based (Table 1).

There are few reports which provide evidence for the role of hygienic design, cooling facilities, sanitation programs and personal hygiene as major measures to prevent microbial food safety hazards (CAC/RCP 1-1969, 2003; CAC/RCP 53, 2003; FDA, 2009; FSA, 2010). However, the impact of changes in climate and global agricultural production trends on the safety of leafy vegetables and the preventive measures and intervention strategies that could be taken at all the

stages of the supply chain have not been thoroughly investigated. The purpose of this review was to collect the key consensus preventive measures and intervention strategies to exclude or, potentially, eliminate pathogens in fresh leafy vegetables along the farm to fork chain including primary production, postharvest handling, processing practices, distribution and consumer handling.

To control, reduce or eliminate microbial food safety hazards of fresh and fresh-cut produce, effective food safety intervention strategies are needed for implementation throughout production, processing and distribution (Luning et al., 2008). Among the intervention strategies included in this review are: (1) *managerial interventions*, which refer to building an operational culture of food safety and commitment to excellence in implementing the preventive control strategies, (2) *intervention equipment* referring to tools and utensils used for the intervention and (3) *intervention methods*, which refer to chemical and physical interventions. Knowing the origin and mechanism of the hazard, the managerial interventions that contribute to controlling microbiological hazards are focused on the functional application of technologies and tactics. We propose this techno-managerial intervention is the strongest strategic approach to deal with problems originating from the complexity and variation of food products and processes.

PRIMARY PRODUCTION

Fresh leafy vegetables are grown and harvested under a wide range of climatic and geographical conditions, using various agricultural inputs and technologies, and on farms or in protected culture of varying sizes. Microbial food safety hazards and sources of contamination may vary significantly by the type of crop, production systems and practices and from one particular setting/context to another, even for the same crop ([FAO/WHO, 2008b](#); [Fan et al., 2009](#); [Sapers et al., 2009](#); [Warriner et al., 2009](#)). The application of hazard analysis and critical control point (HACCP) principles to primary production, while not broadly applicable, is generally feasible as a springboard starting approach to organizing an operational food safety management plan. HACCP, as a formal program is not feasible due to the absence of strict critical control points and the legal burden of some aspects of record-keeping and documentation. A modified approach of hazard analysis and preventive controls (HAPC) together with available and evolving guides to good practice should encourage the use of appropriate hygiene practices at farm level ([EC 852, 2004](#)).

Growing field and adjacent land

Primary production should not be carried out in areas where the known or presumptive presence of pathogens would lead to an unacceptable likelihood of transfer to horticultural crops intended for human consumption without a validated process kill step ([CAC/RCP 1-1969, 2003](#); [CAC/RCP 53, 2003](#)). This preventive measure is not always easy to implement as farmers may not control adjacent land activities or the land history does not include knowledge of the level of pathogens in the soil or time to reduce these to acceptable levels ([Suslow et al., 2003](#); [James,](#)

2006). With increasing populations and high demand for land, fresh produce is often grown in close proximity to urban areas or land used for other types of agriculture, such as livestock production. If vegetables are grown next to an animal-rearing operation, there is a potential for product to become contaminated, directly or indirectly, by animals, run-off, bio-aerosols, or vectors associated with the animal operation such as birds, rodents and flies (Brandl, 2006; Gelting et al., 2011). Similar hazards raise concern for proximity to waste stockpiling and management, composting operations, and run-off from areas of concentrated wildlife populations and urban environments (Keraita et al., 2003). Topographical features of the growing fields and adjacent land should also be considered in a hazard analysis. Preventive measures to avoid contamination coming from growing field and adjacent land include the development of risk assessment to identify potential point and non-point sources (FAO/WHO, 2008b). If the growing field is located in a potential hazardous location, intervention strategies focused on the construction of ditches and establishment of buffer areas will help to minimize microbial hazards (Abu-Ashour and Lee, 2000). It is also important to select an adequate crop and crop management practices, including site management that fit a compatible rotation (Leifert et al., 2008).

Animal activity

Contamination of leafy vegetables with pathogenic organisms of human health significance can occur directly or indirectly via animals. Animals can shed foodborne pathogens in the absence of signs of illness, acting as vectors of pathogens such as *E. coli* O157:H7, *Salmonella*, *Listeria* and *Campylobacter* (Moncrief and Bloom, 2005). Domestic animals such as chickens, dogs, cats, and horses can contaminate crops with faecal droppings if they pass through growing areas. However, while domestic animals may be separated from growing operations, wild animals (e.g. frogs, lizards, snakes, rodents, foxes, boars) and birds can only be controlled to a limited extent (Harris et al, 2003; Lowell et al. 2010). Preventive measures such as avoiding access of farm and wild animals to the site and to water sources should be developed and monitored for integrity, particularly near the time of harvest (CCFRA, 2002; CAC/RCP 1-1969, 2003; CAC/RCP 53, 2003). Removing animal attractants and harbourage in the production environment could impact on the animal activity (Thorn et al., 2011). Physical barriers such as mounds, diversion berms, vegetative buffers, and ditches to re-direct or reduce runoff from animal production or waste management operations are sometimes required or a prudent measure (James, 2006). Windbreaks and hedgerows may reduce aerosol drift and attract beneficial but may equally represent habitat for undesirable animals and should be selected and managed accordingly (Lowell et al., 2010). Distress machines and substances, such as those emitting noise or calls (predator calls such as sonic fences and ultrasonic rodent repellents) can reduce animal activity (Caro, 2005). Growers can use scarecrows, reflective strips or gunshots to ward off birds and pests from crops (Fergus, 2011).

Human activity

People working with produce are known to be a source and direct contact vector of microorganisms of significant public health concern. Hygiene practices, from land preparation, planting, weeding, and pruning, to harvest, influence whether produce becomes contaminated from direct human transfer. Personnel who come directly in contact with fresh produce should be properly trained and advised to follow hygiene and health requirements as preventive measures (CAC/RCP 1-1969, 2003; CAC/RCP 53, 2003). It is recommended to have standard enforceable policies and provide training in sanitation to all employees working in primary production (EC 852, 2004). To support this training hygiene and sanitation facilities should be available to ensure that an appropriate degree of personal hygiene can be maintained (CAC/RCP 1-1969, 2003; CAC/RCP 53, 2003; WGA, 2008). Growers may use portable toilet facilities that are placed in appropriate locations for workers in large fields to provide sanitary facilities. If human activity is the reason for contamination, interventions aimed to control microbial risk will be necessary. People known, or suspected, to be suffering from, or to be a carrier of a disease or illness likely to be transmitted through fresh leafy vegetables should not be allowed to enter any food handling area (FAO/WHO, 2008b). Any person affected should immediately report illness or symptoms of illness to the management. If a worker has a potential source of contamination such as cuts or wounds, the cut or wound should be covered by suitable waterproof dressings (WGA, 2008; Ritenour, 2010).

Climatic conditions of the growing area and climate change

The impact of climate change on agriculture has been related to variations in the seasons, modifications of areas suitable for growing crops, alterations of crop yields and changes in soil quality, such as an increase in losses of soil minerals, variation in their bioavailability and alteration in soil microorganism ecosystems (CCSP, 2008; Miraglia et al., 2009). Climate change has been related to changing disaster risk patterns mainly by the increase in frequency and intensity of extreme events (IPCC, 2007). Climate change has been identified as having potential for increasing bacterial contamination of food and water and variation in levels of certain pathogens in agricultural land and water with extreme weather events such as alternating periods of floods and droughts (Rose et al., 2001; Tirado et al., 2010). An increase in frequency and severity of extreme precipitation events may lead to contamination of soil, agricultural lands, ground or surface water and food with pathogens originating from sewage, agriculture, urban, industrial settings as well as flooding events and tailing ponds (IPCC, 2007). Because of compaction, heavy rainfall after drought can result in more severe run-off and an increased risk of certain types of contamination (Abu-Ashour and Lee, 2000). Temperature variations may also affect the safety of the food chain on the basis of changing survival or multiplication of some food-borne pathogens (FAO, 2008). For instance, increased water and air temperatures could stimulate an increase in growth potential or the dissemination of harmful pathogens (D'Souza et al., 2004; Kovats et al., 2005). In addition, climate change can affect infection of crops by toxigenic fungi, the growth of these fungi and the production of mycotoxins (FAO, 2008).

Principles of Good Agricultural Practices (GAP) and Good Handling Practices (GHP) remain the prerequisite cornerstones of food safety management strategies to address challenges posed

by climate change. Production areas should be evaluated for hazards. If the evaluation concludes that contamination in a specific area is at levels that may compromise the safety of crops, in the event of heavy rainfall and flooding for example, intervention strategies should be managed to prohibit growers from the use of this land for primary production. Among the potential interventions, both water treatment and efficient gullies and drain systems that take up the bigger amount of overflow are needed to prevent the additional dissemination of contaminated water (FAO/WHO, 2008b). Other intervention strategies proposed by Tirado et al. (2010) include: (i) use selected crops that are suitable for growth in areas most affected by droughts or floods, saline soils etc. and (ii) new filtration devices based on developments in nanotechnologies that can remove a range of chemical and microbiological contaminants from soils and water and eventually from foods, and (iii) when climate conditions favour the contamination of a specific area or a crop, rapid detection methods of pathogens and microbiological contaminants (mycotoxins) should be used to delimit the contaminated zone.

Equipment associated with growing and harvesting

Equipment and tools used during growing and harvesting should be maintained in good condition. Growers and harvesters must always follow the technical specifications recommended by the equipment manufacturers for their proper usage and maintenance (IFPA, 2001). Equipment and tools should function according to the use for which they are designed without damaging the produce (Giесе, 1991). The Code of Hygiene Practice for Fresh Fruits and Vegetables establishes several sanitary practices that might be considered as preventive measures to avoid contamination in equipment associated with growing and harvesting (CAC/RCP 1-1969,

2003; CAC/RCP 53, 2003). Developing and implementing appropriate cleaning, sanitizing, storage, and handling procedures of all food contact surfaces including harvest containers, tools and harvest implements, belts, sorting tables, other equipment, and packing materials are preventive measures (Stevenson and Bernard, 1999; IFPA, 2001; FDA/CFSAN, 2008; WGA, 2008). Appropriate cleaning system and concentration of the chemicals used for decontamination should be selected (Katsuyama and Strachan, 1980). Sanitation Standard Operating Procedures (SSOPs) establish the frequency of equipment cleaning and sanitation (FDA/CFSAN, 2008). Establishing policies and sanitary design options that facilitate frequent and thorough cleaning and sanitizing of food contact surfaces are also preventive measures needed to provide optimal effort to ensure the safety of leafy vegetables (Marriott, 1989). Interventions to reduce or eliminate contamination through equipment associated with growing and harvesting include the identification of specific hygiene and maintenance requirements for each piece of equipment that is used and the type of fruit or vegetable associated with it (Marriott, 1994; FDA/CFSAN, 2008). Intervention strategies should be managed to discard equipment and tools that can no longer be kept in a hygienic condition. Also, disinfection systems should be in place to ensure proper attention to routine cleaning and maintenance tasks. Sanitation verification methods such as ATP (adenosine triphosphate) and periodic microbiological verification swabbing should be used for harvest equipment (Kottferová et al., 2003). Cleaning of contaminated containers to control, reduce or eliminate microbial risks should be a consistent operational practice. Segregation of contaminated equipment or otherwise identification of contaminated equipment is an intervention strategy to avoid use as harvesting containers (Giese, 1991). In addition, Clean-Out-of-Place (COP) and Clean-In-Place (CIP) techniques to disinfect contaminated equipment are

recommended (Le Gentil et al., 2010). In some cases, brushes, brooms, squeegees, scouring pads, water hoses or sponges may be used as well as mechanical sprayers to increase the force of the cleaning solution against contaminated surfaces (FDA/CFSAN, 2008). Acid and alkaline-based foam detergents are recommended to eliminate biofilms. The application of a detergent solution such as clinging foam, steam and hot water to decontaminate equipment surfaces is also useful (Schmidt, 2009).

Manure and soil amendments

Organic fertilizers such as animal manure may introduce faecal pathogenic bacteria, viruses and parasites to fresh produce if manure is not adequately aged or otherwise treated before application (Mawdsley et al., 1995). Additionally, manure piles stored next to growing operations may represent a risk of contamination also be a problem because of run-off, vertebrate and insect vectors, or aerosols (Suslow et al., 2003; Brandl, 2006; James, 2006). Some of the factors that affect survival of the pathogen in manure-amended soils and the associated risk of pathogen transfer to the produce are: the manure type, management during stockpiling, method of application, application rate, frequency of application and time period between application and planting or harvesting (Strauch, 1977; Suslow, 2001a). Modification of cattle diet, to reduce pathogen concentrations in manure, has been reported as a preventive measure, though results are highly variable among studies and, therefore, controversial in the formation of policies (Franz et al., 2008; Lowe et al., 2010). In some studies, cattle fed with hay produce less *E. coli* counts found in the faeces than those with grain (Diez-Gonzalez et al. 1998). Gilbert et al. (2005) showed a decrease in *E. coli* counts in cattle faeces when fed roughage compared to grain.

Recent studies have identified the impact of cattle diet on both the occurrence of ‘super-shedder’ animals and longevity of persistence of *E. coli* O157:H7 in manure pats even when initial numbers per gram are low (Jacobs et al. 2010). Probiotics such as *Lactobacillus* strains have been also shown to reduce the density of *E. coli* O157 in manure up to 63% (Stephens et al., 2007). Greater attention must be focused on the microbial safety of chicken pellets and other types of non-synthetic soil amendments. Drying the fresh chicken litter/manure at 250 °C eliminated *Salmonella*, fecal streptococci, and enterobacteria (Lopez-Mosquera et al., 2008). However, assumptions cannot be made that these products are safe to use simply because they have been through a thermal process or that the process was properly managed and finished pellets protected from re-contamination (Matthews, 2009).

Proper storage and management of manure, including aerobic composting, anaerobic digestion, aeration of sludge, and stabilization is recommended to reduce residual pathogen population (Suslow et al., 2003; Erickson et al., 2010). Some of the treatment procedures to reduce or eliminate pathogens from contaminated manure are for example: proper composting, pasteurization, heat drying, UV irradiation, alkali digestion, sand drying or a combination of these (CAC/RCP 1-1969, 2003; CAC/RCP 53, 2003; FDA/CFSAN, 2008). For example, proper composting of animal manure via thermal treatment has been described as an effective preventive measure (CFA, 2007; USDA, 2008; Erickson et al., 2010). The pathogen-reduction criteria includes a temperature of at least 55°C for 3 consecutive days in an aerated pile or 55 °C for 2 weeks in the hot zones of a window pile with 5 turnings (James, 2006). This process can kill nearly all-pathogenic microbes and still maintain the population of beneficial ones. Equipment to foster the right time and temperature process used during the composting process

of contaminated manure should be used to monitor and document the process parameters of each batch. The recording of physical (pH, temperature, oxygen, carbon dioxide, ammonia, moisture content) and chemical (fibre content, nutrient status) characteristics of a compost process can be used as a preventive measure to make an estimation of the progress of pathogen reduction of manure and the safety and stability end-point of material considered for application (FAO/WHO, 2008b). In addition to the benefits of pathogen elimination, composting benefits the environment because manure nutrients, especially nitrogen and phosphates are converted to more stable forms and are less likely to reach groundwater or move in surface run-off (Feng et al., 2000).

Soil amendment application techniques must control, reduce or eliminate the likely contamination of surface water and/or edible crops being grown (EFSA, 2005; FAO /WHO, 2008b; WGA, 2008). Close proximity to on-farm stacking of manure should be avoided. If the potential for contamination from the adjoining land is identified, intervention strategies (e.g. care during application and run-off controls) should be implemented to reduce the risk. Control of run-off or leaching by securing areas where manure is stored should be carried out. The proximity of wind-dispersed or aerosolized sources of contamination should be also minimized. Implementing management plans, which ensure that the use of soil amendments does not pose a significant potential for human pathogen hazards, must be considered (FAPQDCP GAP, 2010). Efficient preventive measures to minimize risk include the establishment of suitable conservative pre-plant intervals, which should be appropriate for specific regional and field conditions (Suslow et al., 2003). Effective means of equipment cleaning and sanitation to eliminate contaminated manure are needed. Standard Operating Procedures (SOPs) to prevent cross-contamination of finished compost with raw materials through equipment, run-off, or wind

should be considered (WGA, 2008). Direct or indirect contact between manure and fresh leafy greens should always be minimized while the time interval between the soil amendment application and time to harvest should be maximized. Pre-harvest intervals of 120 days are generally accepted in GAPs guidance though 60 days is considered the minimum duration (Erickson et al., 2010).

Water for primary production (water source and irrigation system)

The external and, possibly, internal contamination of leafy greens by contaminated irrigation water has been frequently reviewed and cited as a major potential risk factor (Suslow et al. 2003; Brandl, 2006; Sapers et al., 2006; Doyle and Erickson, 2008; Hanning et al. 2009; Suslow, 2010; Pachepsky et al., 2011) The selection of appropriate irrigation sources as a preventive measure is very important, avoiding if possible, uncontrolled sources of water such as rivers and lakes. In the case of surface water, interventions to reduce contamination from animals, as well as control run-off are indispensable (Charatan, 1999; Oron et al., 2001; Suslow et al., 2003; Gerba, 2009; Jones and Shortt, 2010; Pachepsky et al. 2011). Additional protection of water sources from seepage is needed where water supplies are delivered in peri-urban or mixed agricultural areas. Sanitary surveys of canals, and ditches should focus on the integrity of surrounding bank systems focusing on potential point source and non-point source confluences (e.g. drainage into these systems) (Jones and Shortt, 2010). Also as a preventive measure, growers should have the water they use periodically tested for microbial and chemical contaminants. The frequency of testing will depend on the water source and the risks of environmental contamination including

intermittent or temporary contamination (e.g. heavy rain, flooding, etc.) (Gerba, 2009; Jones and Shortt, 2010).

Several strategies have been proposed to reduce the risk of produce contamination with pathogens during irrigation (Pachepsky et al., 2011). A decrease of pathogen inflow from direct input sources (runoff, direct deposition, infiltration and lateral flow in shallow soils, sewage discharge) and/or from pathogen reservoirs (bottom sediment, bank soils, algae, and periphyton) presents a possible strategy for microbiological water quality control. Treating water during storage, between storage and delivery systems and while in the delivery systems represents another class of strategies. Water treatments are coagulation, flocculation, filtration, and disinfection. Solar irradiation is also suggested as a contributor to reduction in the levels of pathogenic microorganisms (Caslake et al., 2004). Other intervention strategies have been considered to improve microbial quality of surface wastewaters, such as sand filtration or storage in catchments or reservoirs to achieve partial biological treatment before use (Carr et al., 2004). Manipulating irrigation schedules and concurrent use of irrigation waters of different quality can help to reduce the risk of produce contamination with pathogens. In some EU countries, only general instructions apply to the primary production and operators who are required to use potable or clean water (EC 852, 2004).

Changing the irrigation method may affect the pathogen availability to plants. (Stine et al. 2005a,b). Special attention to the water quality should be considered when using delivery techniques (e.g. sprayers) that expose the edible portion of leafy vegetables directly to water, especially close to harvest time (CAC/RCP 1-1969, 2003; CAC/RCP 53, 2003; Marites et al., 2010; Suslow, 2010). Drip irrigation and well-maintained furrow irrigation, limits contamination

of leaf surfaces (Qadir, 2008). Plants grown in hydroponic systems have been shown to have lower microbial contamination than plants grown in soil (Selma et al., 2012). Water used in hydroponic culture should be changed frequently, or if recycled, interventions such as the use of disinfection technologies are required to minimize microbial and chemical contamination. Irrigation distribution networks are designed to meet peak demands, which might create in some parts of the network low-flow conditions that can contribute to the deterioration of microbial water quality (Chambers et al., 2004). The control of water quality in intermittent supplies represents a significant challenge, because the risk of backflow increases significantly due to reduced pressure. Preventive measures to maintain microbial quality include maintaining disinfectant residual concentrations within a locally predetermined range and minimizing the transit time (WHO, 2004). The use of disinfectant treatments can also be considered as cost-effective intervention strategies that are employed to reduce or eliminate contamination of agricultural water. Disinfectant treatments of surface or well water include chlorination, pH shock, peroxyacetic acid, electrochemical disinfection and UV treatment. Ozonation and chlorine dioxide injection has also been described as a possible disinfection treatment for irrigation water (Suslow, 2004, 2010).

Production systems

Modern agriculture is generally divided into two main production systems: “conventional” and “alternative” systems (e.g. regional, organic and integrated production) (Stefanelli et al., 2010). Current evidence suggests that alternative production systems can be as safe as conventional agriculture depending on specific practices (Selma et al., 2012); however, the

absence of highly efficacious antimicrobials, use of non-composted manure and application of preharvest amendments such as manure teas, compost teas, and application of non-synthetic fertilizers (fish emulsion, algal extracts, liquid green waste extract) through surface water irrigation commonly used in organic food production, may make this type of agriculture theoretically more prone to microbial contamination risks ([Suslow et al., 2001a](#); [Cencič and Borec, 2004](#); [Selma et al., 2010](#); [Tomás-Callejas et al., 2011](#)). Manure and soils should be evaluated for hazards and if the recognized pathogenic microbes of public health importance are present, the use of the interventions mentioned above in the specific sections should be required.

Preventive measures include the use of agricultural chemicals or biological controls that are authorized for the cultivation of the specific fruit or vegetable and to use them according to the manufacturer's instructions for the intended purpose ([CAC/RCP 1-1969, 2003](#); [FDA/CFSAN, 2008](#)). When leafy vegetables are grown indoors (tunnels, and greenhouses), structures should be located, designed and constructed to avoid contamination and harbouring pests such as insects, rodents and birds ([CAC/RCP 53, 2003](#)). Each establishment should be evaluated individually in order to identify specific hygiene requirements for each product ([EC 852, 2004](#)). Intervention strategies aimed at inactivating or eliminating pathogens in order to reduce them to acceptable levels are the use of Integrated Pest Management (IPM) and bio-control measures for pest and disease control, to take measures to exclude domestic animals and wildlife from crops, to treat manure properly to destroy pathogens and stabilize nutrients and to test and remediate (if necessary) irrigation water quality at regular intervals ([Suslow et al., 2003](#); [Islam et al., 2004](#); [Pachepsky et al., 2011](#)).

Preharvest pathogen testing

The approaches, practices, and issues surrounding routine testing of fields for the presence of human pathogens, typically within 5-7 days of harvest, or in the postharvest handling interval as incoming raw materials and/or finished goods is beyond the scope of this article. However, it deserves mention here as an increasingly required activity and expectation for supplier qualification under many foodservice and retail customer sourcing requirements (D'Lima and Suslow, 2009; United Fresh, 2010). Preharvest testing is more common than testing of finished product for processed leafy greens as adequate cold storage to implement a *Test to Release* (Test and Hold) program is generally not available (D'Lima and Suslow, 2009). Current preharvest programs generally include rapid PCR-based tests for *E. coli* O157:H7, non-O157 EHEC (enterohaemorrhagic *E. coli*), and non-typhoid *Salmonella enterica*. While it is abundantly clear that evidence for pathogen contamination at primary production and either carried forward or introduced in postharvest processing and distribution is detected at a low prevalence, reported to be less than 0.1% of all tested lots of lettuce and leafy greens, the limitations of sampling rather than detection is the greater challenge (ICMSF, 2002; D'Lima and Suslow, 2009). The non-random nature of pathogen distribution in a spinach field, for example, makes full adherence to a statistically valid design for sampling impractical and non-economical to exclude all but the most seriously contaminated fields ($\geq 5\%$ defect rate) with a minimum 95% confidence interval (ICMSF, 2002; Gutiérrez-Rodríguez et al., 2011). The prevailing adage is that one cannot test their way to food safety. Implementation of the preventive controls presented in this article and vigilant monitoring of the primary production area, from preharvest to harvest, for uncontrolled sources of contamination remains the key tool for food safety management and makes the

probability of pathogen detection even more remote if based on current limited and random sampling strategies.

POSTHARVEST HANDLING

This section addresses the activities that occur in the field, namely harvest and field packing. A key characteristic of these operations is that they involve considerable contact between fresh produce and workers (handlers), tools, equipment surfaces, water or ice, and the field environment (soil, dust, insects, etc.) (Beuchat, 1996; Beuchat et al., 2001; Sapers et al., 2006). GAPs, GHPs and Good Manufacturing Practices (GMPs) play an important role in preventing the risk of contamination with food safety hazards during harvest and packing (CAC/RCP 1-1969, 2003; CAC/RCP 53, 2003; Parish et al., 2003; EC 852, 2004).

Primary preparation

Primary preparation includes cleaning, trimming and coring of raw materials (FAO/WHO, 2008b). Mechanical or machine harvest has become increasingly prevalent and provides opportunities for increased surface contact exposure (Fallon et al., 2011). In some countries, fresh-cut processors use field coring and trimming of lettuce (Taormina et al., 2009). This technique removes the wrapper leaves, sprays with a sanitizing wash and bags and boxes the lettuce heads in the field. Cross-contamination of lettuce through contact with workers' hands (or gloves), knives, automated equipment (conveyor belt), and wash water may occur (FSAI, 2001; Matthews, 2009; Fallon et al., 2011). The cut end of the lettuce is laden with nutrients that

support bacterial growth (Brandl, 2008). There is a potential to increase the risk of microbial contamination due to unsanitary handling conditions. Equipment used is designed to facilitate adequate cleaning and sanitation as a preventive measure (FDA, 2008). If re-circulated antimicrobial or antioxidant solutions are used on the cut surface, it is necessary to ensure that they do not become a source of contamination (FDA, 2009; Fallon et al., 2011). There are interventions such as washing and disinfection mitigation technologies currently available, feasible and practical for reducing the levels of pathogenic microorganisms. However, the degree of contamination reduction that can be expected from these technologies is low (McEvoy et al., 2009).

Storage and transportation from the field to the packing facility

The improper handling of product immediately after harvest can compromise the safety of leafy greens. Leafy vegetables can become contaminated during storage and transport (Lund, 1992; Nguyen-The and Carlin, 1994; ICMSF, 1998; Brackett, 1999; Nguyen-The and Carlin, 2000). This is particularly true for baby greens that are harvested into bins for transport to the processing facility. Placing the bins directly onto the soil could result in contaminants contacting the bottom of the bin and subsequently transferred to product during stacking (Matthews, 2009). As a preventive measure, leafy vegetables should be stored and transported using adequate facilities and vehicles that minimize damage and avoid access by pests (CAC/RCP, 2003b). Leafy vegetables with symptoms of decay should be segregated before storage or transport (Cantwell, 1997; Wright, 2004; Brandl, 2008). Agricultural workers should remove as much soil as possible from fresh fruits and vegetables before they are stored or transported (Cantwell and

Kasmire, 2002). Care should be taken to minimize physical damage to crops during this process. Intervention strategies efficient in reducing contamination of leafy vegetables include disinfection of contaminated storage and transportation facilities (FDA, 2008).

Handling prior processing

Proper refrigeration is imperative to cool the product, thereby limiting or preventing growth of the pathogen (Matthews, 2009). Leafy greens must be cooled rapidly as soon as possible (less than 90 minutes) after harvest. Most of leafy greens are generally cooled under forced air (pressure-cooling), hydrocooled or vacuum-cooled but passive storage under refrigeration is still a widely used method. Hydrocooling occurs by showering produce with chilled water and rapidly removing heat. It is usually at least ten times faster than forced-air cooling in removing heat from produce and it can serve as a means of cleaning at the same time (Suslow et al., 2003). Hydrocooling presents a risk of pathogen internalization, as well as external contamination with pathogens. Use of a disinfectant in the water is essential. Vacuum cooling is other common cooling method obtained by placing produce inside a vacuum chamber and applying a vacuum, causing water to evaporate from the produce surface, and hence lowering the produce temperature. It is an effective cooling method for produce with a high surface-to-volume ratio, such as leafy vegetables (Ezeike and Hung, 2009). Water may be sprayed on the produce prior to placing it under vacuum. However, research suggests that the process can promote the infiltration of *E. coli* O157:H7 into lettuce (Li et al., 2008), though this has only been demonstrated in lab-scale systems with controversial methodology. As with hydrocooling, proper water disinfection is essential as most all leafy vegetables are exposed to water during harvest or

postharvest operations. Water, either in liquid phase or in the form of ice, is an efficient vehicle for carrying microorganisms. The quality of the water will determine the quality and safety of the product being packed and it will also be dependent on the stage of the operation (CAC/RCP 53, 2003). Though not optimal primary water for rinsing to remove dirt may be of agricultural grade, whereas, potable water should be used for subsequent rinsing steps (James, 2006). Flume channels for sorting and grading product should be cleaned according to a planned sanitation program as a preventive measure to avoid the build-up of debris from recycled water and avoid product contamination (James, 2006). Where water is recycled, interventions should be taken to treat and maintain the quality of the water to reduce the build-up of microorganisms (FDA, 2008). The treatment process should be effectively monitored and controlled. Control of the sanitary quality of water is technologically feasible but requires strict management of operating practices (Suslow, 1997; López-Gálvez et al., 2010; Luo et al., 2011). Many companies use chlorine or other disinfecting chemicals to control microbial load (Gil et al., 2009). The standard sanitizer is chlorine in the form of sodium hypochlorite granules, tablets or liquid (Suslow, 2001b). The use of other disinfection techniques such as UV-C radiation, ozone, hydrogen peroxide, peroxyacetic acid, etc is also recommended (CAC/RCP 53, 2003; Suslow, 2004; FAO/WHO, 2008a,b; WGA, 2008; FDA, 2009). The levels of antimicrobial agents should be monitored and controlled to ensure that they are maintained at effective concentrations (López-Gálvez et al., 2009). Although adequate filtration is considered a best practice, recycled water may be used with no further treatment, provided its use does not constitute a risk to the safety of fresh fruits and vegetables (e.g. use of water recovered from the final wash to the first wash) (CAC/RCP 1-1969, 2003; CAC/RCP 53, 2003).

Storage and distribution

When product is contaminated with human pathogens, refrigerated temperatures, at which produce should be stored, can be used as an intervention strategy to reduce their proliferation during storage and distribution (CAC/RCP 1-1969, 2003; CAC/RCP 53, 2003). However, refrigeration can actually extend survival of pathogens on the surface of produce relative to ambient conditions with moderate to low relative humidity and pathogenic *Listeria monocytogenes* will continue to grow, slowly, at temperatures above 3.8°C (Rodriguez-Romo and Yousef, 2006). In addition, refrigeration units are thought to spread bacteria and mould throughout warehouses, hence routine servicing of air filters and refrigeration systems is required (James, 2006). As cold air systems blow particulates and mould spores into the air, there is also the risk that pathogens may be spread along with the spores from one pallet or bin to another. Preventive measures to ensure product safety include good hygiene and cleaning practices during storage or transportation (FDA/CFSAN, 2008). Disinfection technologies such as ozone have been also used as intervention strategies to reduce contamination of cooling and storage facilities (Suslow, 2004).

PROCESSING

Fresh-cut processing operations involve the application of several unit operations, which can provide opportunities for cross-contamination whereby a small lot of contaminated product may be responsible for contamination of a large lot (IFPA, 2001; Allende et al., 2004; FDA/CFSAN,

2008). It is recommended that processors ensure that their suppliers (growers, harvesters, packers and distributors) have adopted the principles outlined in the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CAC/RCP 1-1969, 2003; CAC/RCP 53, 2003; Suslow, 2003). Preventive sanitation programs such as GAPs, GMPs and Sanitation Standard Operating Procedures (SSOPs), if properly implemented are likely to minimize the chance of contamination by pathogenic bacteria, viruses and parasites (WGA, 2008).

Reception and inspection of raw materials

Inspection is the first operation on receiving the raw materials, as part of quality control to assess goodness-of-fit to standardized product quality specifications. As a preventive measure, raw materials should be trimmed to remove any damaged, rotten or mouldy material as well inspected to discard product of visibly inferior quality or compromised in some manner relative to food safety risks, such as recent abrasions, punctures, insect damage, or bruising (Nguyen-The and Prunier, 1989). The accepted product may be received in a temperature controlled area or quickly moved, following acceptance, into the appropriate temperature storage room or directly to the processing room (Gil and Selma, 2006). If a vegetable product exhibits signs of chemical or physical contamination or other defects, interventions should focus on the use of equipment for grading, trimming and selection of raw materials to eliminate damaged, spoiled, or potentially hazardous product (FDA/CFSAN, 2008). Visual imaging systems for vegetable inspections are currently available to facilitate this task (Nicolai et al., 2007).

Size reduction

In general, leafy vegetables are mechanically sliced using high-speed machines. Vegetable tissue is damaged when knives are not well maintained (Barry-Ryan and O'Beirne, 1998). One of the most important intervention strategies includes replacing and/or sharpening knives on a regular basis to reduce the damage caused to the product (Bolin and Huxsoll, 1991; CAC, 2003; FDA/CFSAN, 2008). Therefore, the periodic maintenance and cleaning during shifts and daily cleaning and sanitizing of equipment surfaces with careful attention to the cutting equipment is also essential to reduce microbial hazards (Garg et al. 1990; Sapers, 2003).

Washing after cutting

Washing is a very critical part of any produce preparation process especially if a raw, processed fresh produce is sold as “ready-to-eat” (Simons and Sanguansri, 1997; Sapers, 2001; Allende et al., 2008; Gil et al., 2011). An optimum washing system for any prepared vegetable process generally consists of three separate washing stages and three tanks (FSAI, 2001). The first of these tanks aims to eliminate general field dirt and debris. The microbiological load of this wash water increases rapidly; so proper water management is necessary by filtration and refreshing the water, respecting the product/water ratio and application of a disinfectant agent to keep the microbial load of the water at a lower level (López-Gálvez et al., 2010; Holvoet et al., 2011). A flotation washing system, where high volumes of air are blown into the tank through pipes located just beneath the surface of the water, is the preferred solution for products that float (Simons and Sanguansri, 1997; Artés and Allende, 2005). This creates a vigorous Jacuzzi effect, which causes produce to tumble around and creates the mechanical action need for optimal

cleaning. Any accompanying dirt and debris should be loosened and washed off and if such dirt and debris is likely to float, a proper design will incorporate a system to remove it (Turatti, 2011). Dirt and debris that sinks to the bottom of the tank should be released through a periodic drainage system with on-going renewal by fresh water.

In the second tank, the microbiological load is further decreased but the more important function is to minimize cross-contamination during the wash and chill process (Luo et al., 2011). Also here, a proper water management is required i.e. product/water volume ratio, frequency of refreshing the water and application of a disinfectant agent (Holvoet et al., 2011). The sanitation of the product takes place in the second tank in which the water is treated with an agent that is designed to prevent cross-contamination during washing (CCFRA, 2002). The efficacy of sanitizers and other interventions aimed at reducing pathogen levels has been widely considered (FAO/WHO, 2008b). The main effect of sanitizing treatments for washing fresh-cut produce is to reduce the microbial load and keep process water free from contamination rather than having a preservative effect on the produce itself (Baert et al., 2009; Gil et al., 2009). Many studies have been performed on the use of sanitizers, but at European level there is still discussion about their use and each member state needs to approve their use (EC 852, 2004). Among sanitizers, ease of use and relative low cost mean that chlorine is still the most widely used option as a disinfection agent able to prevent pathogen cross-contamination of produce during washing (López-Gálvez et al., 2009; Luo et al., 2011). However, its antimicrobial properties are dependent on the amount of available free chlorine (in the associated form of HOCl) in the solution, the pH, the temperature, and the amount of organic matter (Suslow, 1997, 2001). Oxidation reactions with organic matter and soluble organic and inorganic constituents rapidly deplete free chlorine resulting in an

uncontrolled and widely fluctuating dose in the process water. As a consequence, a new chlorine stabilizer, T-128, has been developed. Although it had low to moderate effects on chlorine stability in the presence of lettuce extract and soils, it significantly reduced the potential of pathogen cross-contamination during produce washing (Nou et al., 2011).

Physical intervention strategies for pathogen inactivation on produce include ionizing irradiation (Farkas, 2006; Fonseca 2006), high pressure processing (Arroyo et al., 1997), high-intensity electric field pulses (Mosquera-Melgar et al., 2008), electrolyzed water (Ongeng et al., 2006) and ultraviolet radiation treatments (Allende et al., 2006). Irradiation has been shown to be effective at reducing enteric pathogens on intact and fresh-cut produce. Ionizing radiation has been shown to greatly reduce potential microbiological risk without damaging the texture/colour of produce (Niemira et al., 2003). For water disinfection, UV light is also a promising technology but its antimicrobial efficacy can be influenced by product composition and soluble solid content of the process water (Allende and Artés, 2003). New UV advanced disinfection technology systems result in a more efficient disinfection as they increase the amount of water that passes close to the UV lamp (Milly et al., 2007; Selma et al., 2008).

Considering the advantages and the disadvantages of the use of chlorine, chlorine derived products have a greater potential for the disinfection of vegetables when suspended or dissolved organic matter are minimized or eliminated. Modern produce washers are designed taking into account interventions to assist with the disinfection process by incorporating different stages such as showers to remove fluids and exudates from cut surfaces before disinfection (Gil et al., 2009). The last stage before packaging should include a final tank stage using non-chlorinated

rinse water to remove traces of sanitizer and reduce produce temperature. Ozone is a frequent choice for treatment of this final rinse water.

Dewatering The time and speed of centrifugation, or alternative dewatering systems, are key parameters to be adjusted for each product. To reduce tissue damage and consequently microbial deterioration in leafy vegetables that are too delicate to withstand centrifugation, intervention strategies such as the use of forced air or air-bed conveyors may be used (Turatti, 2011). Any forced air used in this operation must be filtered to avoid the contamination of the product.

Weighing and packaging

The final operation in the processing of fresh-cut produce takes place in the assembly and packaging room, ideally separated from the washing section. Packing is performed around a vertical tube at the top of which is the associated weight-based portion control machine. Leafy vegetables are collected in the collating funnel that feeds the packaging machine. Packaging under hygienic controlled conditions immediately after drying has an important role for the microbiological protection of fresh-cut produce (FAO/WHO, 2008b; Turatti, 2011). As a preventive measure, operators working in the assembly room must wear protective clothing to avoid contamination of the product (Turatti, 2011). A positive air pressure must be maintained with filtered air (Hurst, 2002). The correct combination of packaging material, produce weight, and gas composition within a package are critical components, which must be determined for each product to maintain product quality and safety and extend product shelf life (e.g. packaging design and materials should provide adequate protection to minimize contamination of produce

and prevent damage) (Jacxsens et al., 2002a; 2002b; Jacxsens et al., 2003). Packaging cannot correct unsanitary product handling, temperature abuse, or poor-quality raw materials. Interventions include the selection of appropriate packaging technologies such as modified atmosphere packaging (MAP) to reduce growth of microbial pathogens (Zagory, 1999).

Storage and distribution

In general, the fate of enteric pathogens on produce during storage is dependent on a number of factors, including storage temperature, relative humidity, gaseous composition of the atmosphere, nutrient availability, and presence of competitive bacteria or antimicrobial compounds (Garg et al., 1993; Zagory, 1999; Doyle and Erickson, 2008). Proper control of temperature during storage and transportation will prevent or delay growth of most microbes on fresh-cut leafy vegetables although it will not eliminate them entirely (Bolin and Huxsoll, 1989; FAO/WHO, 2008b). Thus, the storage unit must maintain the fresh leafy vegetables at appropriate temperatures (AFDO, 2004; Wright, 2004). Temperature and humidity information can be tracked to determine if food products are transported and stored under appropriate conditions (Matthews, 2009). The use of a radio frequency identification device (RFID) would permit the tracking of leafy greens from the field to the retail level. The RFID tags can be continuously written to with information, and read remotely. The RFID system can be monitored via the Internet, and the technology can be used by large and small operations alike. The RFID system can be integrated into Good Manufacturing Practices and Hazard Analysis Critical Control Point systems. In storage, temperature should be monitored by a continuous time-temperature recording device or periodic checks with a calibrated thermometer are suggested

(Yildiz, 1994). Many types of thermometers and temperature recording devices are readily available for use in food handling operations (AFDO, 2004). Thermistors, thermocouples and infrared thermometers are recommended.

If contamination occurs in the storage room, intervention strategies aimed at cleaning and disinfecting the entire facility should be performed to reduce any potential pathogen contamination (IFPA, 2001). Of special importance is that drains, cooling coils, drip pans, ice machines and other areas that are routinely cold and wet are regularly cleaned, sanitized, and swabbed to prevent the survival of microbial foodborne hazards (Korsten and Zagory, 2006).

RETAIL FOOD SERVICE OPERATION AND CONSUMER HANDLING

The fresh-cut segment supplies both the food service industry and retail outlets (Nicola et al., 2009). Approximately 60% of fresh-cut produce ends up in the food service industry, with 40% going to the retail market. Outbreak investigation results indicate that contamination of produce by food service workers has been a significant factor in foodborne illness associated with consumption of fresh produce (NACMCF, 1999). This may be directly related to the significant amount of direct hand contact which occurs in food service and retail establishments during the preparation of fresh produce items for serving. Since the preparation of produce items at food service and retail establishments typically does not involve application of a treatment designed to inactivate microorganisms, food worker hygienic practice can be expected to have a direct influence on the microbiological characteristics of fresh produce items. Retailers should have receipt procedures to identify and accept only fresh produce that meets their specifications. On

the sales floor, produce racks and wet racks should hold food at the ideal temperature and moisture conditions. In wet markets, a typical scenario is the lack of refrigeration facilities. In these cases, an alternative cooling system is still possible e.g. spraying with potable water or alternatives that use very simple evaporative cooling systems (FAO/WHO, 2008b). Employees should clean the racks to assure quality is maintained at its highest level (EC 852, 2004). Retailers and food service operations implement the principles of HACCP to manage food safety in their operations. Guidance documents include a model HACCP plan, best practice guidelines for activities and a sanitary equipment buying guide and development checklist (James, 2006; McClure, 2011). Display involves holding the finished products in temperature control units for a specified duration and condition for public sale (AFDO, 2004). The display unit, including liner or slip sheet and shelving must be clean and well maintained to provide a sanitary environment for product. Workers should be trained in good food-handling practices to ensure that food quality and safety are maintained (EC 852, 2004).

Automated systems are being developed to integrate the whole supply chain from the source to the end consumer. These data-based systems would provide complete and constant visibility of product, package, purchasing and distribution. There are practical technologies that are available to be used by industry, competent authorities or consumers to verify that fresh and fresh-cut produce have been maintained under continual refrigeration (Hofer et al., 2006). The new equipment of RFID technology uses grain-sized computer chips to track items at all points of their journey to the store. Once products arrive at the store, it is possible to track the performance of display cases to assure the product is being held in perfect conditions to maximize quality and safety (Hofer et al., 2006). Though a different type and function for

traceability, another technology known as Microbial Source Tracking uses genetic fingerprinting to identify and link individual or clusters of human cases and sources of foodborne outbreaks rapidly and can genetically match multiple outbreaks that are occurring over long period of time. This technique has been used to trace the pathogenic agent back to its source- all the way to the field where it was grown.

Consumers could be a source of fresh produce contamination in retail outlets. Consumers touch fresh vegetables as they make a decision on whether to purchase product. If a person's hands are contaminated because of improper hygiene, this product could be affected though the scope of the cross-contamination is likely to be highly localized (James, 2006). Vehicle temperature and time of cooling determine the potential for pathogens to multiply when the consumers place grocery bags in the car. At home, food safety practices such as hand washing before handling fresh produce are necessary. Consumers may store vegetables in domestic refrigerators that are designed for general food storage and thus have numerous temperature zones within (FAO/WHO, 2008a; 2008b). The home refrigerator may also harbour harmful organisms if it is not cleaned routinely. Another more serious and common reservoir for pathogenic microorganisms is the kitchen sink, sponges, and dishrags. Consumers may place fresh produce items in the sink without washing or sanitizing the area. This causes cross-contamination from items previously placed in the sink. Consumers may not always wash vegetables, but even the simplest washing with running water is sufficient to cause 1 log reduction in microbes (James, 2006). Fresh-cut vegetables should always be stored in refrigeration, first to extend the shelf life and then to reduce the potential growth of pathogens. In order to improve product safety, the role of consumer education, training and awareness is

considered critical all along the chain. Consumers are an important target group for information and education on how to handle fresh and fresh-cut produce safely, and need to understand their roles and responsibilities in protecting these products from contamination (Hofer et al., 2006). The role of the retailer has to extend beyond the shop. Food safety should not stop once the food goes into the shopping trolley. The retailer should be the best source of food safety information for the consumer. In addition, it is to a retailer's advantage to educate customers about products and food safety. Educating a customer about food safety may retain a customer who shops with confidence and total trust.

CONCLUSIONS

General guidelines to minimize microbial food safety hazards of leafy greens mainly focus on defining preventive measures. However, they often lack specific and practical intervention strategies, which are described or derived more often from a wide range of scientific journals, where their efficiency is demonstrated. The aim of this review was to discuss the preventive measures and intervention strategies from production to processing and consumption of fresh leafy vegetables based on the identified potential microbiological hazards. The primary step to prevent contamination from occurring is to respect the preventive measures included in the GAPs, GMPs, GHPs and SSOPs in primary production, postharvest handling and processing. We encourage growers at the level of primary production to apply HACCP as far as possible.

The preventive measures included in this review were compiled from the guideline documents prepared by governments and farmer and industry associations. These guidance

documents have been complemented with specific information described in dedicated codes of practice of leafy green vegetables. Among the preventive measures and intervention strategies, one of the most important is the training and education of the growers and handlers along the entire food-chain. These training programs include information related to the safe growing and handling practices such as clean handling procedures, control of cross-contamination and personal hygiene. The persistence of existing habits and attitudes may influence compliance to procedures. Numerous intervention strategies to control microbial hazards exist, however, there is no uniquely effective or singular control point. Given the complexity and differences of the primary production, processing, packing and distribution systems, there are intervention strategies that need to be taken at all these steps to control hazards. The differences between large and small-scale production regarding growing, handling and distribution practices should not influence the control of food safety hazards. In addition, changes in processing technologies that can prolong the shelf life of the product should not enable the risk of increased outgrowth of pathogens. More information may be needed on the logistics during distribution and retail sale operations, particularly in relation to time and temperature of storage and distribution of fresh-cut products. It is therefore concluded that there is no unique preventive measure or intervention strategies that could be applied at one point of the food-chain. Thus, dealing with the food safety issues associated with fresh produce it is clear that a “farm to fork” approach is required taking into account a multidisciplinary strategy. Managerial interventions of experts from the food-chain including agronomists, food microbiologists and food science experts are needed.

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Table 1 Codes of practice and guidelines and regulations related to microbiological safety of fresh and fresh-cut products

Title	References
Guide to minimise the microbial food safety hazards for fresh fruits and vegetables. Guidance for industry.	FDA, 1998
Food safety guidelines for the fresh-cut produce industry.	IFPA, 2001
Code of practice for food safety in the fresh produce supply chain in Ireland.	FSAI, 2001
Recommended international code of practice. General principles of food hygiene.	CAC/RCP 1-1969, 2003; CAC/RCP 53 2003
Guidance for processing fresh-cut produce in retail operations.	AFDO, 2004
On the hygiene of foodstuffs.	EC 852, 2004
Recontamination as a source of pathogens in processed foods. A literature review.	ILSI, 2005
On-farm produce standards.	FSLC, 2007
Growing and production of fresh produce: Guidance, developing, documenting, implementing and auditing an SQF 1000 system: level 2.	SQF, 2007
Considering water quality for use in the food industry.	ILSI, 2008.
Commodity specific Food safety guidelines for the production and harvest of lettuce and leafy greens.	WGA, 2008
Microbiological hazards in fresh fruits and vegetables.	FAO/WHO, 2008a
Microbiological hazards in fresh leafy vegetables and herbs.	FAO/WHO, 2008b
Draft Guidance for Industry: Guide to minimize microbial food safety hazards of leafy greens.	FDA, 2009
Monitoring microbial food safety of fresh produce.	FSA, 2010
Evaluation of agronomic practices for mitigation of natural toxins.	ILSI, 2010