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ENVIRONMENTAL AWARENESS AND MANAGEMENT

WORK-ORIENTED TRAINING COURSE

ECOLOGICAL AWARENESS AND MANAGEMENT OF CROSS-KINGDOM PATHOGENS

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LO 11: PLANTS AS VEHICLES FOR THE TRANSMISSION OF FOR CROSS-INFECTIVE PATHOGENS TO PLANTS AND HUMANS

1. Introduction

The number of human infections caused by opportunistic pathogens has increased dramatically in recent years. Each year, roughly 1 in 6 people gets sick from eating contaminated food. Growing awareness that contaminated plants, fresh fruits and vegetables are responsible for a significant proportion of food poisoning with pathogenic microorganisms endorses the demand for understanding the interactions between plants and human pathogens and for determining the specific interventions needed to reduce illness and to measure progress toward public health goals resulting from food safety policies and interventions.

One natural reservoir of opportunistic pathogens is the rhizosphere. While many members of the rhizosphere microbiome are beneficial to plant growth, plant pathogenic microorganisms also colonize the rhizosphere, striving to break through the protective microbial shield and to overcome the innate plant defense mechanisms in order to cause disease. A third group of microorganisms that can be found in the rhizosphere are the true and opportunistic human pathogenic bacteria.

Human pathogens are studied almost exclusively for their detrimental impact on human health. Pathogens possess a diverse set of genetic factors that enable pathogenicity, ranging from specialized secretion systems to toxins and adhesins, all of which are involved in manipulating or circumventing the human immune system. We often consider these human pathogenic bacteria to be dedicated animal pathogens that cause disease and epidemics; yet, the constant interaction of human carriers with their environment predisposes these pathogens to alternative niches that include non-animal hosts. Not surprisingly, evidence is being accumulated that some animal pathogens which are known to cause serious human diseases can also act as plant pathogens. The epidemiology and disease strategies of these pathogens, whether considered primarily a plant or a human pathogen, are of particular interest from the perspectives of both the biology and evolution of cross-kingdom pathogenesis.

The disease incidence related to *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes* infections caused by consumption of (fresh) vegetables, sprouts, and occasionally fruits made clear that these pathogens are not only transmitted to humans via the "classical" routes of meat, eggs, and dairy products, but can also be transmitted to humans via plants or products derived from plants. It is now of major concern that these human pathogens become adapted to environmental habitats without losing their virulence to humans. Pathogen contamination of fresh produce may originate before or after harvest and once contaminated, the produce is difficult to sanitize. This problem is compounded if the bacteria invade the vascular system of the plants, as these bacteria are then protected from any conventional surface sanitation treatments applied. Such treatments reduce the overall microbial load and reduce the levels of surface contamination, but do not penetrate into the plant tissue. Adaptation to the plant environment would lead to longer persistence in plants, increasing their chances of transmission to humans via consumption of plant-derived food.

Numerous pathogenic bacteria appear to have a fairly broad spectrum of host organisms. These cross-infective microorganisms are more insidious than those simply transmitted to humans by contact or consumption of infected plants. The cross-kingdom human pathogens, and their potential plant reservoirs, have important implications for the emergence of infectious diseases. Management of cross-infective diseases of both plants and animals will require more interdisciplinary research and cooperative agency interactions. Estimates of foodborne illness source attribution are used for many purposes, including informing strategic planning, informing risk-based decision-making, estimating benefits of interventions, and evaluating the impact of interventions. The strategies for reducing the contamination from farm to table are high on the agenda of food safety agencies.

This lecture describes the fitness of human enteric pathogens on plants which have implications for food safety. The growth and survival of enteric pathogens on plants are discussed in the light of concepts in plant microbial ecology. Information regarding the various factors that affect the survival of enteric pathogens provides a good foundation for assessing their role in the infectious dose of the pathogens and constitutes a basis for development of a Food Plant Pathogen Awareness Program.

2. Insights into cross-kingdom plant pathogenic bacteria

Life on Earth is diverse and interactive: not one ecosystem harboring a single species has yet been described. Interactions between organisms can be very diverse in nature, ranging from beneficial through neutral to detrimental. An infectious disease is usually defined as the result of a detrimental (set of) interaction(s), and is usually restricted to a given combination of host (the diseased) and pathogen (the disease-causing agent). The infectious disease may be defined as a biological process leading to the disruption of the normal physiology of a multicellular organism in response to the presence of a pathogenic microorganism on or in the body.

Diseases may differ in their presentation but infections are always preceded by adhesion, invasion and colonization. Interestingly, several pathogenic microorganisms are capable of infecting a variety of organisms, and in some cases even members of different biological kingdoms of life may be susceptible. Such cross-kingdom host jumps are defined when a microorganism normally colonizing a species from one taxonomic kingdom becomes able to repeatedly infect a species belonging to another kingdom. Microbial pathogen host jumps occurring at across-kingdom level are not as well-known as those occurring within the animal kingdom, such as those associated with human diseases, but several cross-kingdom host jumps have been described and have highlighted the ability of seemingly dedicated human pathogens to cause plant disease, and specialized plant pathogens to cause human disease. The phrase "human pathogens on plants" (**HPOPs**) has been proposed recently to describe such pathogens when they inhabit, colonize, enter, or otherwise interact with plants.

The disease strategies used by cross-kingdom pathogens to infect unrelated hosts are of particular interest, since plant and animal (human) hosts have distinctive physical barriers and defense responses. While specialists may have evolved dedicated factors for overcoming the physical barriers and innate defenses in a certain host, a cross-kingdom pathogen would require a diverse library of genes and disease strategies to overcome the specific defense obstacles of each of its hosts. This would either necessitate a suite of pathogenicity factors to enable attachment, disease development, and dispersal for each host, or a universal disease strategy in which the same suite of pathogenicity factors is used for all hosts. Phytopathogens utilize specific determinants that help to breach reinforced cell walls and manipulate plant physiology to facilitate the disease process, while human pathogens use determinants for exploiting mammalian physiology and overcoming highly developed adaptive immune responses. Potential cross-kingdom pathogenic microorganisms must be able to come into close and frequent contact with potential hosts, and must be able to overcome or evade host defences. Reproduction on, in, or near the new host will ensure the transmission or release of successful genotypes. Signatures of host adaptation will be apparent in these determinants, which may include incremental mutations and genetic rearrangements, along with the acquisition of novel genetic elements that may contribute to virulence or host specificity. The identification of these genetic determinants in cross-kingdom pathogens with both human- and plant-pathogenic potential can provide a better understanding of the evolution of phytopathogenicity, as well as the role of plants as potential reservoirs for clinically relevant bacteria (Kirzinger et al, 2011).

An unexpectedly high number of cross-kingdom host shifts of bacterial and fungal pathogens have been described. The Gram-negative bacterial family *Enterobacteriaceae*, which includes many human pathogens (e.g., *Escherichia*, *Salmonella*, and *Shigella*) associated with plant foods, also contains a number of genera of plant pathogens (*Enterobacter*, *Erwinia*, *Pantoea*, *Pectobacterium*, etc.), which cause plant diseases such as blights, wilts, and soft rots. The taxonomic relatedness of these plant and human pathogens raises interesting questions about the possibilities for niche competition or synergism, horizontal nucleic acid exchange in protected plant niches, or even host range expansion.

There are microbial species, sometimes referred to as **cross-over pathogens**, that infect and cause disease on both plants and humans, though these are relatively uncommon. Examples of currently recognized **cross-kingdom pathogens** include a few bacterial species that commonly inhabit plant surfaces and the rhizosphere, such as *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Dickeya* spp., *Enterococcus faecalis, Serratia marcescens*, *Agrobacterium tumefaciens*, *Phytophthora infestans*, *Salmonella enterica*, *Escherichia coli,* and *Listeria monocytogenes.*

2.1 The plant environment as a habitat for human pathogens

Agricultural plants have become a source of human pathogens, especially emerging ones that belong to the group of Shiga toxin-producing *Escherichia coli* (STEC) strains. The threat of human pathogens in freshly consumable products of plant origin became apparent during the outbreak caused by *E.coli* O104:H4 in Germany and France in 2011, where almost 4000 persons became infected leading to 54 casualties and over 900 incidences of hemolytic uremic syndrome. The most likely transmission route of the pathogen to consumable products was remarkable as the source was Fenugreek seeds that were transported from Egypt to Rotterdam harbor, the Netherlands, approximately 17 months before appearance of the first disease incidences in Hamburg and the surrounding area. Although the pathogen was neither found in Fenugreek sprouts, nor in the seeds themselves, epidemiological facts revealed that the pathogen must have been associated with seeds over a relatively long period of time.

This raised the questions on how a human pathogen can persist as a viable entity in a hostile environment for such a long period and why similar observations had not been made before. Must this be considered as the first incidence of a disease outbreak caused by a human pathogenic bacterium that was adapted to the plant environment? To address this question, it must be referred to the nature of the causative agent that is atypical of pathogenic *E. coli* strains commonly occurring in Europe and the USA. The *E. coli* O104: H4 outbreak strain was an enteroaggregative strain that, unlike other typical *E. coli* O type strains, does not have animals as a major reservoir, but only humans instead. Outbreaks caused by this type of pathogen are rare in Western societies, whereas those caused by *E. coli* O157:H7 and *S. enterica* are more common. This indicates that particular features in these human pathogens already exist, extending their life-time in the plant environment. The question is whether these features were intrinsic to particular subsets of human pathogens or were recently gained, e.g., via horizontal gene transfer. The strain that caused the outbreak in the Hamburg area must be considered as a highly virulent pathogen and it must have acquired its virulence and antibiotic resistance traits via horizontal gene transfer events like transduction (phage infection) and conjugation (plasmid transfer). Acquisition of new virulence traits is one aspect in the evolution of a new pathogen, but selection pressure is another, and the outbreak strain must have evolved by increasing its virulence in humans side-by-side with improvement of its ecological competence in plants. The threat of this development is the emergence of new types of highly virulent human bacterial pathogens that are fully adapted to life near, or maybe inside agricultural plants.

2.2 Human pathogens that can infect plants

Our human-centric view of disease often leads to sweeping assumptions that human pathogenic bacteria are devoted to human hosts. Species of *Salmonella*, *Serratia*, *Enterobacter*, and *Enterococcus* are commonly considered problematic human pathogens that are frequently found in the nosocomial environment, and which cause food poisoning, general infections, and septicaemia. Actually, other bacterial species that cause skin, wound, and urinary tract infections (e.g. *Bacillus cereus*, *Proteus vulgaris*) can also be found in rhizosphere environments.

Relatively recent studies, however, have begun to uncover that these human-pathogenic bacterial species are also capable of colonizing and causing disease in a wide variety of plant hosts. Various studies have indicated that human pathogenic bacteria enter the root tissue at sites of lateral root emergence. Notably, many of these studies have been conducted under laboratory conditions, providing evidence for the phytopathogenic potential of these bacterial species. However, the incidence of plant disease caused by many of these human pathogens in the natural environment remains unknown.

The occurrence of human pathogenic bacteria in the rhizosphere has been ascribed to several factors, including the high nutritional content, protection from ultraviolet (UV) radiation, and the availability of water films for dispersal and for preventing desiccation. Others have argued that the abundant and highly diverse indigenous rhizosphere microbial communities provide a strong barrier against the invasion of human pathogens. For example, the growth of *S. enterica* and *E. coli* O157:H7 on roots of *Arabidopsis thaliana* was strongly inhibited by a plant-associated strain of *Enterobacter asburiae*. Nevertheless, many human pathogenic bacteria can be highly competitive for nutrients and produce various antimicrobial metabolites allowing them to colonize and proliferate on plant surfaces in the presence of the indigenous microbial communities. Interestingly, the mechanisms involved in rhizosphere colonization and antimicrobial activity of human pathogenic bacteria appear to be similar to those involved in virulence and colonization of human tissues.

Next, we will discuss the biology and pathogenesis of some of the most common causative agents of infection associated with the consumption of plant foods.

2.2.1. *Salmonella*

This genus is composed of two species; *Salmonella enterica* and *Salmonella bongori*. *S. enterica*, subdivided into hundreds of serovars, is the causal agent of many human, animal, and bird diseases worldwide, including gastroenteritis and typhoid fever. It is the pathogen most frequently linked to consumption of fruit and vegetables. Despite its clear adaptation to survival in human hosts, *Salmonella* has also been detected in the rhizosphere of several crop plants. For a long time, *Salmonella* was assumed to survive on plants after a more or less accidental infection. However, this notion has recently been challenged. *Salmonella* bacteria are able to persist in soil. Studies on native *Conzattia multiflora*, a legume tree indigenous to Mexico, revealed the existence of *Salmonella* plant-borne lines isolated from nodule-like structures. *S. enterica* ssp. *enterica* serovar Typhimurium (*S. typhimurium*) has been found in the rhizosphere of several crop plants including: wheat (*Triticum sativum*), oilseed rape (*Brassica napus*), and strawberry (*Fragaria ananassa*). *S. enterica* levels on wild tomato (*Solanum pimpinellifolium*) were lower than on domesticated tomato cultivars (*Solanum lycopersicum*). An additional infection route could be water, which was in contact with human or animal waste. Contaminated water is a well-known means of dissemination of numerous pathogens, including *Salmonella*. In this way, bacteria can move through the water supply system and subsequently reach crop fields where the contaminated water is used for irrigation. *S. enterica* populations were bigger in the phyllosphere of tomato plants irrigated with contaminated water than in plants grown from seeds in pre-infested soil. Infection of plants with *Salmonella* may also occur with the help of other organisms. Co-inoculation with the nematode *Caenorhabditis elegans* and *Salmonella* Newport of lettuce, strawberries or carrots placed on top of animal or composted manure resulted in plant infection with these bacteria; conversely, the plant tissues were not infected with the pathogen in the absence of the nematode. *Salmonella* can also be transported via insects, e.g. by the pharaoh ant (*Monomorium pharaonis*), moving from indoor facilities to the environment, and therefore coming in contact with a range of possible hosts, including plants. The interaction between *Salmonella* and arbuscular mycorrhzal fungi (AMF) has demonstrated a higher persistence of *Salmonella* in plants colonized by AMF, which indicates another layer of interactions in the rhizosphere.

The susceptibility to contamination by *S. enterica* via soil differs among agricultural crops. Members of the *Brassicaceae* family presented higher bacterial populations than tomato and lettuce. However, lettuce was shown to have a more highly contaminated phyllosphere, suggesting that other contamination routes, such as irrigation water, are also effective. Another noteworthy possibility is that the plant itself might contribute to infection. A study performed on alfalfa sprouts demonstrated that, during seed germination, the endosperm breakdown causes the release of reducing sugars and other organic molecules, which can be found in the irrigation water and, in consequence, produce a growth medium for *S. enterica*. Since bacteria metabolize these molecules as a source of nutrients, plant exudates seem to be a suitable nutrient source. The high multiplication rates of some *Salmonella* strains in effluxes of germinating seeds has prompted the suggestion of saprophytic growth. Furthermore, other plant pathogenic bacteria may contribute to the infection with *Salmonella*. Pectinolytic bacterial pathogens, which cause soft rot and in this way mobilize nutrients, are frequently associated with infection of fruits and vegetables by *S. enterica*. *Salmonella* is not only capable of colonizing the phyllosphere; it has been shown to infect the plant and cause death of plant organs. Inoculation of *Salmonella* in *Arabidopsis* via shoot or root tissues resulted in chlorosis, wilting, and eventually death of the infected tissues within seven days.

2.2.2. *Escherichia coli*

Escherichia coli is a bacterium that is commonly found in the gastrointestinal tract of humans and warm-blooded animals. Due to its high prevalence in the gut, *E. coli* is used as the preferred indicator to detect and measure faecal contamination in the assessment of food and water safety. Considered as harmless commensals, *E. coli* strains constitute about 1 percent of the normal gut microbial population. While most of the strains within the gut are beneficial for human gastrointestinal function, others are harmful. Pathogenic *E. coli* strains are distinguished from others by their ability to cause serious illness as a result of their genetic elements for toxin production, adhesion to and invasion of host cells, interference with cellular metabolism and tissue destruction. To be transmitted from one host to another, these bacteria must leave their host and get into the surrounding environment. There is evidence that some *E. coli* can survive for several weeks outside the host, and even grow in water or soil. But it is on plant matter that *E. coli* colonization has become a concern, since although most types of *E. coli* are harmless, the presence of pathogenic strains on fruit and vegetables presents a food safety risk. Produce-associated outbreaks of *E. coli* O157 infections linked to consumption of leafy green vegetables have been increasingly recognized. However, several *E. coli* clones have acquired pathogenicity islands via horizontal gene transfer that enable them to cause urinary tract infections and diarrheal diseases. Diarrheal *E. coli* are divided into six categories that can cause illness ranging from moderate diarrhea to severe systemic diseases (e.g. hemolytic-uremic syndrome).

Shiga toxin-producing *E. coli*

Shiga toxin-producing *E. coli* (STEC) is a zoonotic pathogen colonizing mainly cattle and small ruminants. Although cattle products, principally beef, are the most commonly recognized sources of *E. coli* O157 infections, fruits and vegetables consumed raw are also an important source. Three leaf attachment mechanisms have been described in *E. coli* O157. First, contrary to nonpathogenic *E. coli*, the pathogenic strain STEC O157:H7 adheres strongly to tomato skin, spinach leaves, and roots of alfalfa sprouts. Adhesion to these surfaces is mediated by curli. The adhesion of *E. coli* O157, as well as the related enteropathogenic *E. coli* (EPEC), to a variety of salad leaves is mediated by the filamentous type secretion system (T3SS). The *E. coli* O157 secretion system is designed to translocate effector proteins into mammalian cells; protein translocation is mediated by an ATPase and is dependent on a translocation pore that is inserted into the plasma membrane. The flagella also play a role in *E. coli* O157 leaf attachment. Taken together, these data suggest that *E. coli* O157 employ multiple mechanisms to colonize plants and are well adapted to this biosphere. Like *Salmonella*, *E. coli* O157 can also reach the sub-stomatal cavity and the spongy mesophyll and survive in this environment.

Enteroaggregative *E. coli* and enterotoxigenic *E. coli*

Enteroaggregative *E. coli* (EAEC) may be an important cause of bacterial gastroenteritis. However, the reservoir of EAEC is unknown. There have been observed the following two patterns of bacterial distribution following 1 h incubation of EAEC with lettuce leaves: (i) diffuse adherence to the epidermis and (ii) localized adhesion to the guard cell of the stomata. The binding to the epidermis is mediated by the pilus, which is known to play a role in colonization of the human gut, whereas aggregation around the stomata is mediated by flagellae.

Enterotoxigenic *E. coli* (ETEC) is an important cause of infantile and travelers' diarrhea and causes severe watery diarrhea in calves and piglets. The flagellum is the main adhesin mediating the attachment of ETEC to the epidermis of lettuce leaves.

2.2.3. *Serratia marcescens*

Members of the genus *Serratia* cause important infections in humans, animals, and insects. Taxonomically, the genus *Serratia* is confusing, and currently there are 14 recognized species, with 2 subspecies. The type species, *Serratia marcescens*, is an opportunistic human pathogen commonly found in the respiratory and urinary tracts of humans, and is responsible for approximately 1.4% of nosocomial infections that can be life-threatening. While environmental *S. marcescens* strains are often red due to the production of prodigiosin, the strains associated with hospital outbreaks are mostly non-pigmented. As many *S. marcescens* strains are also resistant to multiple antibiotics, it represents a growing problem for public health. However, relatively little is known about the factors that contribute to *S. marcescens* pathogenesis within its host.

Yet, despite its animal virulence factors, *Serratia* has also been found to be a common phytopathogen. *S. marcescens* is recognized as a phloem-resident pathogen that causes cucurbit yellow vine disease of pumpkin (*Cucurbita moschata* L.), watermelon, and yellow squash (*Cucurbita pepo* L.), which is marked by wilting, phloem discoloration, and yellowing of foliage. *S. marcescens* produces a biofilm along the sides of the phloem vessels, blocking the transport of nutrients and eventually causing the plant to wilt and die (Sikora et al., 2012).

2.2.4. *Enterobacter cloacae*

Another bacterium that has also shown cross-kingdom pathogenesis is the Gram-negative bacterium *Enterobacter cloacae.* It is an important nosocomial pathogen responsible for bacteremia, lower respiratory tract infections, skin, and soft-tissue infections, as well as urinary tract infections. *E. cloacae* synthesizes a Shiga-like toxin II-related cytotoxin, which has been implicated in an infant case of haemolytic-uremic syndrome. Although there is evidence that *E. cloacae* has evolved to colonize the human host, it has also been identified as the causal agent of grey kernel disease of macadamia (*Macadamia integrifolia*). The onset of grey kernel disease not only affects the quality of the kernels produced by the tree, but also results in grey discoloration and a foul odor. *E. cloacae* also causes bacterial soft rot disease in dragon fruit (*Hylocereus* spp.), bacterial leaf rot in *Odontioda orchids,* and is also responsible for internal yellowing disease in papaya and onions. Enterobacter bulb decay develops after onions are harvested, cured, and stored. The decay usually occurs in a few scales of the onion bulb and the tissue develops a brown color giving the bulb a *dirty ring* appearance when cut in half. If storage lots of onions have a high enough incidence of Enterobacter bulb decay (>2–5%), the whole lot cannot be sold and results in a significant loss to the grower. The mechanism of how *E. cloacae* causes bulb decay, as well as the specificity of the virulence factors employed in each of these hosts are unknown and, as a result, the development of disease control methods for bulb decay are limited.

2.2.5. *Enterococcus*

Cross-kingdom pathogenesis is not limited to Gram-negative bacteria. Enterococci are part of the normal intestinal flora of humans and animals, but are also important pathogens responsible for serious infections, especially in immunocompromised patients. With increasing antibiotic resistance, enterococci are recognized as nosocomial pathogens that can be challenging to treat. The genus *Enterococcus* includes more than 17 species, but only a few can cause local or systemic clinical infections, including urinary tract and abdominal infections, wound infections, bacteremia, and endocarditis. Clinical isolates of *E. faecalis* have been found to produce hemolysin—a virulence factor that leads to the lysis of red blood cells. Hemolytic strains exhibit multiple drug resistance more frequently than non-hemolytic strains, whereas strains isolated from fecal specimens of healthy individuals display a low (17%) incidence of hemolysin production. *E. faecalis* is not only capable of infecting mammalian and nematode hosts, but also the plant *Arabidopsis thaliana*, causing plant death seven days after inoculation under laboratory conditions. The manifestation of disease initially begins once the bacterium has successfully attached itself to the leaf surface, where upon entry into the leaf tissue through the stomata or wounds, *E. faecalis* multiplies and colonizes the intercellular spaces of the plant host and causes rotting and disruption of the plant cell wall and membrane structures. The phytopathogenicity of *E. faecalis* appears to involve some of the same genetic determinants involved in animal pathogenesis, including the quorum-sensing system gene and a serine protease. *E. faecalis* clearly uses a general disease strategy that allows it to use the same virulence factors for exploiting two different hosts.

2.2.6. *Listeria monocytogenes*

L. monocytogenes is best known as a serious human pathogen that is transmitted to humans via consumption of contaminated food products. However, it is also a bacterium that occurs commonly in soils, associated with decaying plant matter. Thus, it can be naturally associated with any plant product grown in contact with soil. Unusually for human pathogens, it can use plant sugars for growth and hence, the organism is often a problem associated with minimally processed fruit or vegetables. Specifically, when vegetables or fruit are cut, sugars are released which the organism can utilize for growth.

***Listeria* in plant tissue.** It is still unclear whether *L. monocytogenes* can become internalized in plant tissue. The information currently available on the internalization of *Listeria* is from very limited studies and some of the results are contradictory. It has been shown that, like *E.*

coli, *Listeria* can enter the stomata of leaves but whether this is an active process or a passive entrapment of bacteria within the structure (which is far larger than the bacterial cell) has not been determined. However, bacteria able to enter natural openings in the plant can be protected from various sanitizers following closure of the guard cells. Since the organism is commonly found in soil, the entry of *Listeria* into leaf tissue is more likely to occur post-harvest rather than during growth. The fruit and vegetable crops can be contaminated post-harvest when there is a big enough temperature differential between the crop and wash water to allow movement of water into the plant tissue. Following entry, it is likely that the bacteria are transported via the mass flow of water entering the plant and moving within it.

The exception to this is when *Listeria*-contaminated manure has been used as a fertilizer, as occurred in the Maritime coleslaw outbreak in 1981. This was the first outbreak of *L. monocytogenes* that could be definitely linked to food and was caused by commercially prepared coleslaw in Canada and resulted in at least 41 cases and 7 confirmed deaths. In this case, it was found that manure from *Listeria*-infected sheep had been used as an organic fertilizer when growing the cabbages used to prepare the salad, leading to heavy leaf contamination.

Growth of *Listeria* on fresh produce. Produce commodities can differ in their ability to support growth of *L. monocytogenes*. Some studies have been carried out to compare growth on intact and damaged or cut produce. Growth has been reported on intact tomatoes, but studies on carrots, cabbage, peppers, and cantaloupe melon reported growth only on cut, shredded or damaged produce. In general, *Listeria* grows well on shredded lettuce, with populations increasing during storage. Survival and growth patterns on vegetables have been found to vary with strain, product type, and packaging atmosphere. It has been suggested that packaging under modified atmosphere increases the ability of *L. monocytogenes* to grow by inhibiting competing microflora, but this remains to be proven, and the effect may differ by commodity, atmosphere composition, and storage conditions. However, modified atmosphere packaging (MAP) evidently does not inhibit the growth of *L. monocytogenes*, and the prolonged shelf life may in itself increase the listeriosis risk.

Significant differences have been observed in terms of the ability of *Listeria* to survive in heat and acidic conditions and on packaged vegetables. Cooked or pasteurized produce usually supports rapid growth and may represent a risk after post-processing contamination, especially if not refrigerated and consumed without heating. It is now accepted that most produce commodities support the growth of *L. monocytogenes*.

3. Conclusions

- The number of outbreaks of foodborne disease linked to the consumption of fresh produce has increased since the early 1990s.
- *S. enterica* and *E. coli* O157:H7, both previously associated with illness from foods of animal origin, cause the highest proportion of fresh produce-linked epidemics that have a known etiologic agent.
- Surveillance data have provided strong evidence for the presence of enteropathogenic bacteria on fresh fruit and vegetables.
- Not all enteric pathogen species are ecological generalists. Large differences exist among various enteric pathogens in their ability to attach to and colonize plant surfaces, and particular phenotypes play a role in some of these differences.
- Although the fitness of *S. enterica* on plants is relatively weaker than that of common plant-associated bacteria, this human pathogen can grow on plant surfaces under conditions of high moisture and warm temperature, two factors that affect its competitiveness in that habitat.
- Several studies performed in growth chambers and in the field have demonstrated that *S. enterica* and *E. coli* inoculated at planting time persist on crops for prolonged periods of time, including until harvest.
- Diseased plant tissue may provide a nutrient-rich and protected ecological niche for enteric pathogens. However, this opportunity for growth is dictated by the nature of their interactions with the resident plant microflora.
- The expression in enteric pathogens, of survival determinants or of virulence traits in the plant habitat, may modulate the dose–response relationships in the human host and allow for foodborne illness to occur with low infectious doses.

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LO 12: FOOD SAFETY, PLANT PATHOGEN AWARENESS AND MANAGEMENT

1. Introduction

Evidence is emerging that enteropathogenic bacteria have the ability to grow and persist on crop plants. This versatile lifestyle may explain the remarkable incidence of foodborne illnesses associated with the contamination of agricultural crops. It could be argued that the size of the enteric pathogen population on produce does not constitute the sole determinant of infectivity in the human host. The interactions of enteric pathogens on plants may enhance not only their survival in that habitat, but also their ability to infect humans.

Food safety has major implications for human health, social behavior, and economy. Food-borne disease outbreaks can make a substantial number of people ill, while attendant recalls and publicity can reduce consumer confidence and decrease demand with significant economic loss for all parts of the supply chain. For these reasons the Food plant safety Awareness Program was developed. There are three steps used to manage health and the safety of plant products: 1) spot the hazard (hazard identification); 2) assess the risk (risk assessment); 3) make the changes (risk control).

2. Key risk factors affecting pathogen infection and spread

The risk factors for produce contamination are in the supply chain, both at the pre-harvest (in the field) and post-harvest stages. During the pre-harvest phase, pathogen populations can establish themselves on growing crops. The risk can be amplified after harvest either by further direct contamination, or by proliferation of existing pathogen populations during processing and post-harvest handling procedures.

Water is likely to be an important source of contamination in the field. Possible sources are run-off from nearby animal pastures and irrigation from a contaminated source. The risk associated with using water from a range of sources that vary in microbiological quality for irrigation of produce has been assessed and the need for improved guidelines has been recognized. While studies have found no internalization of *E. coli* O157:H7 in spinach plants through contaminated soil, uptake and internalization was found in spinach leaves after contaminated water was dropped on the leaves. This suggests a lower likelihood of transmitting pathogens from contaminated water through drip irrigation versus a higher likelihood through overhead sprinkler systems. However, irrigation is not the only reported route of contamination linked to water. Use of water in post-harvest processing has also played a role. An outbreak of infections with *Salmonella* serovar Newport was linked to consumption of mangoes treated with a process involving hot water aimed at preventing importation of fruit flies. Pathogens may be transferred to the environment by application of inadequately composted or raw animal manures or sewage. The faeces of wild animals may also be a source. In the US outbreak of *E. coli* O157 infections associated with pre-packaged spinach, trace-back and environmental investigations determined that one ranch in California's SalinasValley was the likely source of the outbreak. The patterns produced by pulsed-field gel electrophoresis (PFGE) and multilocus variable number tandem repeat analysis (MLVA) of the strains involved in the outbreak matched those from isolates recovered from local feral swine and cattle faeces. However, the manner in which the spinach became contaminated was not determined. Insects are also a possible source of contamination. In laboratory conditions, contaminated flies have been shown to directly transfer bacteria to plant leaves or fruits. Studies have implicated flies in contamination of leaves by *E. coli* O157:H7. Large numbers of flies belonging to the Muscidae and Calliphoridae families which were found in production fields adjacent to rangeland habitats occupied by cattle were shown to carry *E. coli* O157:H. Proposed sources of contamination of fruit used for juice have included the use of fallen fruit that has been in contact with contaminated soil, water, sewage or manure, use of contaminated water in washing or processing of fruit, and contamination at the point of consumption. Post harvesting processes, ranging from storage and rinsing to cutting, are also possible sources of contamination. Cut surfaces of leaves are a specific target for pathogenic bacteria such as *Salmonella*, which show a specific tropism towards them, and cutting melons may carry pathogens from the rind onto the edible part of the fruit where bacteria may multiply if the cut melon is not refrigerated. The use of inadequately decontaminated water in hydrocoolers, which are used to store and process large quantities of fresh produce, can lead to contamination of an entire lot.

Other multiple factors that have likely contributed to the emergence of produce as a source of enteric illness are:

- Changes in the produce industry:
    - Intensification and centralization of production;
    - Wider distribution of produce over longer distances;
    - Introduction of minimally processed produce;
    - Increased importation of fresh produce;
- Changes in consumer habits:

- Increased consumption of meals outside the home;
- Increased popularity of salad bars;
- Increased consumption of fresh fruits and vegetables, and fresh fruit juices;

➢ Increased size of at-risk population (elderly, immunocompromised people);
➢ Enhanced epidemiological surveillance;
➢ Improved methods for detection, identification and tracking of pathogens;
➢ Emerging pathogens with low infectious dose.

3. Sanitary risk analysis in plant products food safety schemes

The risk assessment work conducted on the risk associated with plant products has found that fresh cut fruit and vegetables, seed spouts, vegetables in oil, and unpasteurized juice present a high risk. This was done due to a history of foodborne illness outbreaks in Australia and the USA, mainly due to *Salmonella*. Annual consumption of these products in Australia has been estimated as being 11,000 tonnes of fresh cut vegetables, 150 tonnes of fresh cut fruits, 2600 tonnes of seed sprouts, 1000 tonnes of vegetables in oil products, and 100,000 L of unpasteurized juice.

Surveys of plant products have shown the potential for these high-risk plant products to be contaminated with *L. monocytogenes*, *Aeromonas* spp., *B. Cereus*, and *Salmonella*. Contamination of fresh cut fruit and vegetables can occur during growth, harvest or processing, with the main pathogens of concern being *L. monocytogenes* in general, and *C. botulinum* for products packaged under modified atmosphere. These products are considered high risk when they are consumed raw. Seed sprouts can become contaminated with *B. cereus*, *Salmonella*, and pathogenic *E. coli* during growth and harvest of the seeds and also during the sprouting process, which provides a near perfect environment for the growth of microorganisms. The oxygen-reduced environment provided by vegetables immersed in oil allows for the growth of anaerobic microorganisms, including *C. botulinum*, the cause of botulism. To reduce the risk, the vegetables or fruits are usually cooked and acidified prior to placement in oil. Unpasteurized fruit juices may become contaminated during the juicing process, either due to contamination on the exterior of the fruit, or the use of damaged and mouldy fruit. Because the juice is not heat treated, any pathogenic microorganisms present are able to survive, and acid-tolerant strains of pathogenic *E. coli* and *Salmonella* may grow.

3.1. Vulnerable persons food safety scheme

Certain population sub-groups are more at risk of foodborne illness or can develop more severe conditions due to foodborne illness when compared to the general population. The degree of vulnerability depends on the susceptibility of the individual and the pathogenicity of the pathogenic microorganism. In general terms, the vulnerable population group includes children under five years of age, people over 65 years, pregnant women and persons with depressed immunity.

It is estimated that the number of meals served to vulnerable persons in different facilities in New South Wales (Australia), such as hospitals, aged-care facilities, hospices, day care establishments, and childcare centres, is approximately 133 million meals per year. It is estimated that up to one million meals per year served at these facilities may be contaminated with a foodborne pathogen.

Since 1995, there have been 65 foodborne illness outbreaks in Australian aged-care facilities, childcare centres, and hospitals, with 758 illnesses and 75 fatalities. The pathogens implicated have included *Salmonella*, *C. perfringens*, *L. Monocytogenes*, and *Campylobacter*. The prevalence of foodborne-related illness and deaths in the elderly living in nursing homes is far greater than the baseline level of illness in the general population, while children appear more at risk to *Salmonella* due to high salmonellosis rates in children seen both in Australia and overseas. The major hazard of concern to vulnerable persons is *L. monocytogenes*, with some sub-groups within the vulnerable population being 100 times more susceptible to listeriosis than the general population. Other hazards of concern include infants exposed to *C. botulinum* through consumption of contaminated honey, neonatal infants consuming *Cronobacter sakazakii* (formerly *Enterobacter sakazakii*) contaminated infant formula, and individuals with liver dysfunction exposed to *Vibrio vulnificus* via raw oysters. Other organisms that may result in more severe illness in vulnerable sub-groups include pathogenic enterohaemorragic *E. coli*, *S. aureus* and *C. perfringens*.

When assessing the risk associated with foods, it is important to consider food preparation and hazardous scenarios. Businesses catering to vulnerable persons need to consider the susceptibility of their consumers when designing menus and sourcing, preparing and serving foods. Under the Food Standards Code, these establishments are required to implement a food safety program including substitution of high-risk foods with lower-risk alternatives, effective cleaning and sanitation of fruits and vegetables to be consumed raw, limiting the storage, ensuring that foods are cooked properly, and effective cleaning and sanitation of equipment.

3.2. Risk assessment

Risk assessment is the rational application of safety principles to available options for handling hazardous materials. The following characteristics are considered when evaluating a potential pathogen:

- the agent's biological and physical nature;
- the sources likely to harbor the agent;
- the host susceptibility;
- the procedures that may disseminate the agent;
- the best method to effectively inactivate the agent.

The biological nature of pathogens determined their distribution **into risk groups.**

Risk groups of pathogens

Microorganisms that are human pathogens can be categorized into risk groups (RG) based on the transmissibility, invasiveness, virulence (i.e., ability to cause disease), and the lethality of the specific pathogen. Risk groupings of infectious agents (RG1 through RG4) approximately correspond to biosafety levels (BSL1 through BSL4), which describe containment practices, safety equipment, and facility design features recommended for safe handling of these microorganisms.

Beginning with RG1 agents, which are nonpathogenic for healthy human adults, the scheme ascends in order of increasing hazard to RG4.

RISK GROUP 1 agents are not associated with disease in healthy adult humans. Examples: *E. coli* K-12, *Saccharomyces cerevisiae.*

RISK GROUP 2 agents are associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available. Examples: enteropathogenic *E. coli* strains, *Salmonella*, *L. monocytogenes*, *Cryptosporidium*, and *Staphylococcus aureus.*

RISK GROUP 3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk). Examples: human immunodeficiency virus, *Brucella abortus*, *Mycobacterium tuberculosis*.

RISK GROUP 4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk). Examples: *Ebola* virus, *Cercopithecine herpesvirus* 1 (Herpes B or Monkey B virus).

Consideration of the risk group assignment, however, merely is a starting point for the comprehensive risk assessment. Further attention must be given to the circumstances, such as the planned procedures and the available safety equipment. Then, the recommended precautions may be increased or decreased relative to those based solely on the risk group assignment and adjusted to reflect the specific situation in which the pathogen will be used. Microorganisms in RG1 require use of standard basic biological laboratory facilities and microbiological practices, whereas those in RG4 require maximum containment facilities and practices. Some of the agents likely to be handled experimentally at UW-Madison are RG2 or RG3 pathogens; designated as moderate and high hazard, respectively. These agents typically require more sophisticated engineering controls (e.g., facilities and equipment) than are available in standard laboratories, as well as special handling and decontamination procedures. Consideration also is extended to microorganisms that cause diseases in animals and/or plants, which are not categorized into risk groups as are human pathogens. The desired containment for animal and plant pathogens is based on the severity of the disease and its ability to disseminate and become established in the local environment.

The progression from invasion to infection to disease following contact with an infectious agent depends upon the dose, route of transmission, invasive characteristics of the agent, virulence and resistance of the exposed host. Not all contacts result in infection and even fewer develop into clinical disease. Even when disease occurs, its severity can vary considerably. Attenuated strains should be handled with the same precautions as the virulent strain unless the reduced pathogenicity is well documented and is irreversible. Viral vectors, even if rendered replication defective, still may pose a threat of recombination with wild-type strains and/or unintentional delivery of their foreign genes. It is prudent to assume virulence.

Which human pathogens are harbored by plant products?

Four priority pathogens have been identified by the regulatory agencies as the initial focus of foodborne illness source attribution work: *Salmonella*, *E. coli* O157:H7, *Listeria monocytogenes*, and *Campylobacter*. Furthermore, surveys of plant products have shown the potential for high-risk plant products to be also contaminated with *L. monocytogenes*, *Aeromonas* spp., *E. coli*, *B. cereus*, *C. botulinum*, and *Salmonella*, when they are consumed raw. Contamination of fresh cut fruit and vegetables, as discussed in the previous section, can occur during growth, harvest or processing and storage of harvest and packing in a modified atmosphere.

The presented risk assessment will review the hazards associated with food businesses regulated under the food safety schemes of Food Regulation and includes plant products, such as fresh cut fruits and vegetables, unpasteurized juice, and vegetables in oil. Risk assessment forms part of an overall process, called risk analysis. Risk analysis is used by governments and industry to assess, manage, and communicate the risk associated with particular food or food groups and in turn aims to reduce the risk of foodborne illness.

The Codex Alimentarius Commission (CAC) divides **risk analysis** into **three components**:

• **Risk assessment** – a process by which the potential risk posed by food safety hazard(s) is determined;

• **Risk management** – the process of determining alternatives to control the hazards identified in the risk assessment; and

• **Risk communication** – the exchange of information on risk and risk management amongst interested parties.

CAC (1999) has identified **four components of risk assessment**:

• **Hazard identification** – the process of identifying potential hazards associated with the food.

• **Exposure assessment** – an estimation of the potential human exposure to the hazard, which includes the use of data such as the occurrence in a particular food and/or potential consumption rates of the food. Exposure assessment can be defined as the set of circumstances that influence the extent of exposure.

• **Hazard characterisation** – the evaluation of the potential illness associated with the hazard.

• **Risk characterisation** – the process of determining the probability of occurrence and severity of the adverse health effects based on the information collected in the hazard identification, exposure assessment and hazard characterisation. Risk characterization can be viewed as a "quantitative measurement" of the probability of adverse effects under defined conditions of exposure. Some authors, when evaluating the effect of plant pathogens, also add the step of dose-response evaluation, which involves determination of the relationship between the magnitude of exposure and probability of the adverse effect.

3.2.1. Hazard identification

Microbiological hazard was designated for six products (Table 1) with high risk of contamination with pathogens. The food safety scheme was developed to introduce minimum regulatory requirements for businesses producing high-risk plant products, and to implement control measures to minimize the risks from the microbiological hazards associated with these products.

Table 1. Microbiological hazards associated with plant products

Plant product	High risk ranking	Medium risk ranking
Fresh cut vegetables – may be consumed raw	Pathogenic *E. coli* *Salmonella* serovars *L. monocytogenes*	
Fresh cut vegetables – chilled, MAP or extended shelf life	*L. monocytogenes* *C. botulinum*	
Vegetables in oil	*C. botulinum*	
Seed sprouts	Pathogenic *E. coli* *Salmonella* serovars	*B. cereus* *L. monocytogenes*
Fresh cut fruit	Pathogenic *E. coli* *Salmonella* serovars *L. monocytogenes*	*Cryptosporidium parvum* Enteric viruses
Fruit juice / drink (unpasteurized)	*Salmonella* serovars Pathogenic *E. coli*	

Adapted from FSA (2000a)

Fresh cut vegetables

Fresh cut fruits and vegetables are raw agricultural products that have been processed by means of washing, trimming, cutting or slicing to be made ready for consumption. Contamination of vegetables may occur during growth, harvest or processing. Under certain conditions microorganisms can also become internalized within the vegetables. Conditions that promote internalisation of microorganisms include damage to the natural structure (e.g. punctures, stem scars, cuts, and splits) and placing warm produce into cooler, contaminated wash water. These foods have a high potential risk due to the contamination with pathogenic *E. coli*, *Salmonella* serovars and *L. monocytogenes*. The actual process of cutting and/or removing the protective outer surfaces of the plants may increase the potential for pathogenic bacteria to survive and/or grow. Many vegetable products do not undergo a kill step that will completely eliminate pathogens; however, measures such as sanitising washes may be used to reduce microbial contamination with pathogens.

Many fresh cut vegetables are packaged using MAP and refrigerated to extend the shelf life. This form of processing may lead to an increased risk from pathogens such as *L. monocytogenes* and psychrotrophic strains of *C. botulinum* by enhancing the conditions for their survival and allowing additional time for growth. MAP products may become fully anaerobic if the plant tissue is actively respiring and uses up all the oxygen. As any competition from aerobic spoilage organisms is inhibited, this may increase the opportunity for anaerobic or facultative anaerobic pathogens to grow.

Fresh cut fruit

Fresh fruit are normally perceived as low risk foods, as they tend to have a thicker protective skin than most vegetables and most are harvested from trees or bushes. The notable exceptions are melons and strawberries, which are considered higher risk because they grow close to the ground and their surfaces may become contaminated with soil.

Contamination of fruit may occur at any point from growing (soil, fertilisation, irrigation water, and animal/bird waste), through harvesting and processing (including washing), to distribution, marketing, and consumption. Many microbial pathogens cannot survive or grow on most fruit due to the low pH environment. However, melons and strawberries have relatively high pH, which makes them more likely to be a food safety hazard. In addition, the skin of rockmelon tends to be porous, which may allow the penetration of pathogens and agricultural chemicals into the fruit. Melons are often dipped in a sanitizing solution after harvest (FSA, 2000a).

Fresh cut fruits may be value added by peeling, chopping, slicing, and packaging. Many fresh cut fruit are packaged using MAP and refrigerated to extend the shelf life. With additional time, this can lead to an increased risk from pathogens that are adapted to the acidic environment of fruit and are able to survive and grow in these foods.

A range of bacterial (*E. coli*, *Salmonella* serovars, and *L. monocytogenes*) and viral pathogens, and enteric parasites (*Cryptosporidium parvum*) have been identified as being of concern in fresh cut fruit. The actual process of cutting and/or removing protective outer surfaces of the fruit may increase the potential for pathogens to survive and/or grow. Fruit pickers and handlers with infections are also an important source of contamination.

Vegetables in oil

This product category includes a diverse range of vegetables and mixtures of vegetables and herbs that may be used fresh, dried, roasted or acidified. Oil is added to exclude air, which prevents discoloration of the vegetable. Although immersion of vegetables in oil reduces the available oxygen in the container, contrary to popular belief, it does not preserve the food. Some pathogenic bacteria are able to survive and grow in reduced levels of oxygen and even under anaerobic conditions. *C. botulinum* is the main pathogen of concern because of its ability to grow anaerobically and it has been linked to outbreaks of illness from the consumption of vegetables in oil. Vegetables may be contaminated by *C. botulinum* spores, which are frequently associated with soil, and processes such as cooking and acidification may be insufficient to inactivate the spores or prevent their germination and growth. Acidification to below pH 4.6 should prevent outgrowth; however, more than one hurdle is recommended as a safeguard.

Seed sprouts

Seed sprouts are usually consumed raw and include alfalfa, mung bean, chickpeas, cress, fenugreek, soy, lentils, sunflower, onion, and radish. Seeds for sprouting generally do not receive any special treatment during harvesting and transport, and so may become contaminated with pathogenic organisms in the field or during harvesting, handling, processing, and distribution. While some bean sprouts may be cooked prior to consumption, many others are consumed raw, for instance with salads.

The microbiological pathogens frequently found associated with seeds for sprouting include *B. cereus*, *Salmonella serovars*, and *E. coli* and these organisms have also been implicated in foodborne illness outbreaks. The rough surfaces and cracks in the seed may protect the pathogens from microbiocidal treatments and may make detection during routine analysis difficult. High levels of organic matter also reduce the effectiveness of chlorine treatments during seed washing and seed sprouting. Bacterial populations of 10^2–10^7 cfu/g have been observed on seeds for sprouting, and this natural population can rapidly increase under the high moisture and moderate temperature conditions used in sprouting facilities. Microorganisms may also become internalized in the sprout during growth, so sanitising wash treatments of sprouted seeds are not likely to be effective.

Unpasteurized fruit juice

Fruit juices are made by extracting fruit (citrus juices) or by macerating fruit (grape, cherry, berry, apple juice, etc). This may be followed by clarification, filtration, pasteurisation, and/or other processes to reduce the microbial load. In recent years, there has been a trend to produce ‘natural’ fruit juices containing no preservatives and receiving little or no heat treatment.

Any microorganisms present on the surface of fruit may potentially contaminate the juice made from it. Bacterial pathogens are unlikely to grow due to the low pH but some bacteria, viruses or protozoa may be able to survive for extended periods. The length of time the microorganism may survive is dependent on the pH of the juice, the storage temperature, and the physiological state of the microorganism. Some *Salmonella* serovars and strains of pathogenic *E. coli* are known to be particularly acid tolerant, with this response thought to be activated by previous exposure to sub-lethal pH values.

Apple and pear juice can become contaminated by the mycotoxin patulin, which is produced by several *Penicillium* and *Aspergillus* species. *P. expansum* appears to be the main patulin producer in apples and apple products. Since patulin is concentrated in the rotting tissue of fruit, it is a good indicator of the quality of fruit used to make the juice.

The acidic nature of fruit juices makes them corrosive to metals. To avoid potential chemical contamination, only stainless steel or corrosion resistant vessels should be used to store these products. Other metals such as copper can leach into the beverage during storage.

3.2.2. Exposure assessment

Production data

Leafy salad vegetables, such as lettuce, rocket, and baby spinach, are the most common products in the fresh cut category. Based on limited industry information, the estimated annual consumption of fresh cut fruit and vegetables is: 11,000 tonnes of fresh cut vegetables, 150 tonnes of fresh cut fruit, approximately 1000 tonnes of vegetables in oil, and between 2100 and 2600 tonnes of seed sprouts. Fruit juice suppliers suggest that manufacture of unpasteurized fruit juices occurs at relatively low volume, about 100,000 L/year, not including juices prepared in retail premises.

Consumption of plant products

Consumption of fruits, during the period 1997–98 and 1998–99, increased by 8.3% from 124.7 kg per capita to 135.0 kg. In the same period, the import of oranges and other citrus fruit rose by more than 62%. Consumption of vegetables has shown a steady 9.4% increase over the last decade. Per capita consumption of tomatoes showed a significant increase from 20.9 kg in 1997–98 to 24.9 kg in 1998–99, a rise of 19%. The category of other vegetables showed a 4.6% increase in consumption in 1998–99 to 25.1 kg per person. Approximately 35% of all respondents consumed fruit juices and drinks, with the mean consumption being 250 mL / day.

Prevalence of hazards in plant products

Generally, there have been a small number of surveys of plant products. The analysis of 54 samples of ready-to-eat salads and vegetables showed that only one was positive for *L. monocytogenes*. Szabo et al. (2000) tested 120 minimally processed, cut and packaged lettuce samples. Three samples (2.5%) were positive for *L. monocytogenes*, 66 samples (55%) were positive for *Aeromonas hydrophila* or *A. caviae* and 71 samples (59%) were positive for *Y. enterocolitica*. The survey of the microbiological quality of freshly squeezed juices showed that *L. monocytogenes* was detected in 1/291 samples (0.3%), but the level was sufficient to classify the sample as potentially hazardous. *E. coli* was detected in 7/291 samples (2.4%). In 2006, it was found that *E. coli* was detected in 7 out of 261 samples (2.7%) of sprouted seeds from a retail store, while *Listeria* and *Salmonella* were not detected in any samples. A sample was found to be potentially hazardous due to the presence of verotoxigenic *E. coli* (VTEC) and a further two samples were categorized as unsatisfactory due to elevated levels of *E. coli*. A more extensive survey of 122 samples in 2008 found that 99.2% of samples were microbiologically acceptable, with a single sample categorized as unsatisfactory due to *B. cereus* at a level of 5500 cfu/g. *E. coli* was detected in 1/119 samples (0.8%) of fresh cut vegetables, while *Salmonella*, *L. monocytogenes* and VTEC were not detected in any samples. The Food Safety Authority of Ireland (FSAI) surveyed the bacteriological safety of a range of plant products as part of a European Commission coordinated program (FSAI, 2003). Pre-cut fruit and vegetables had samples classed as unacceptable/potentially hazardous due to the presence of *Salmonella* in 1/529 samples (0.2%) and *L. monocytogenes* in 1/344 samples (0.3%). Qualitative tests found 21/513 samples (4.1%) positive for *L. monocytogenes*. No sprouted seeds samples were classed as unacceptable or potentially hazardous. *L. monocytogenes* was detected in 1/26 samples (3.8%). No problems were detected with unpasteurized fruit and vegetable juices.

A similar European Commission program surveyed pre-packed mixed salads from retail premises in the UK for *L. monocytogenes*. *L. monocytogenes* was detected in 4.8% of collected samples. A parallel survey by FSAI included *Salmonella* testing in the survey design. Quantitative analysis detected two samples with *L. monocytogenes* at levels exceeding 100 cfu/g. Summarized microbiological results of surveys of prepared salads and fruit examined in the UK showed no isolations of *E. coli* O157 or *Campylobacte*r. Five out of 3852 samples (0.1%) of bagged salad vegetables were positive for *Salmonella* but other commodities were negative. *L. monocytogenes* and *E. coli* were detected in most commodities surveyed, usually at low incidence.

Studies of bacterial pathogens in plant foods in the US (2006 *Annus horribilis*) showed a drastic increase in contaminated food: sprouts, *Salmonella* – 100 cases; lettuce, *E. coli* O121:H19 – 4 cases and *E. coli* O157:H7 – 162 cases; spinach, *E. coli* O157:H7 – 202 cases; carrot juice; *Cl. botulinum* - 6 cases; tomatoes, *Salmonella* – 400 cases; strawberries, suspected *L. monocytogenes*; *c*antaloupes and spinach, suspected *Salmonella.*

3.2.3. Hazard characterisation

Foodborne illness outbreaks from plant products

An indication of the exposure to hazards in plant products is provided by an examination of the foodborne illness outbreaks between 1995 and 2008 attributed to fresh produce and plant products.

Prior to this period, in 1989, in Australia, there were three separate outbreaks from fruit salad due to *Salmonella*; while in 1991, a nationwide outbreak of Norovirus was attributed to the consumption of unpasteurized orange juice. In addition, the risk of listeriosis from plant products was highlighted by an outbreak of listeriosis from contaminated fruit salad. In 1998–1999, six deaths of elderly patients occurred and nine others were affected.

In 1996, an outbreak of *E. coli* O157:H7 infection occurred among schoolchildren in Sakai City, Osaka, Japan. The outbreak was attributed to white radish sprouts served in a centralized luncheon program servicing 56 schools. Over 8000 children developed symptoms and 398 children were hospitalized. Two further incidents of *E. coli* O157:H7 in neighboring areas were also related to white radish sprouts. All the implicated sprouts were traced back to one farm. This illustrates the size of an outbreak that can result when a hazard becomes a reality in a centrally processed and widely distributed product.

In the USA, from 1993 to 1997, there were identified 190 produce-associated outbreaks, resulting in 16,058 illnesses, 598 hospitalisations and eight deaths. The produce-associated outbreaks were an increasing proportion of all reported foodborne outbreaks with a known food cause, rising from 0.7% in the 1970s to 6% in the 1990s. Salad, lettuce, juice, melon, sprouts, and berries constituted the fresh produce most frequently implicated. In the period from 1990 to 2005, there were reported several cases of contamination of greens-based salads, melon, lettuce, and sprouts with *Salmonella*. Produce-related outbreaks resulted in an average of 47.8 cases, which is higher than the incidence reported for outbreaks from poultry, beef, and seafood. Four further outbreaks that occurred in 2006 were detected: an outbreak traced to fresh spinach contaminated with *E. coli* O157; salmonellosis traced to tomatoes; and two outbreaks linked to lettuce contaminated with *E. coli* O157:H7. By January 2007, 205 cases had been reported with 103 hospitalisations, 31 cases of haemolytic uraemic syndrome (HUS), and three deaths confirmed. Contamination was traced back to one farm. While no definitive determination of how the pathogens contaminated the spinach could be made, the presence of wild pigs near the growing fields and the irrigation wells was determined to be an environmental risk factor. Processing of the spinach included washing, but this did not eliminate the problem and may have facilitated the spread of pathogens from contaminated to uncontaminated spinach. This is an example of a widespread outbreak of severe bacterial illness attributable to hygiene failures in the growing and processing of spinach. In 2008, a large outbreak of *Salmonella saintpaul* in the USA and Canada was associated with multiple raw produce items. In August 2008, 1442 people were affected, with at least 286 hospitalisations. Moreover, the outbreak might have contributed to two deaths. The epidemiological data suggested that the major vehicle for the spread of the pathogen was jalapeno peppers. However, serrano peppers were also considered to be a vehicle, and early in the outbreak tomatoes were considered a source. Contamination of produce may have occurred on the farm or during processing or distribution. The outbreak strain of *Salmonella* has been found in one growing area and an associated packing facility in Mexico. This is the largest culture-confirmed outbreak in the USA in the last decade. As many persons with *Salmonella* illness do not seek care or have stool specimens tested, many more unreported illnesses may have occurred.

In England and Wales, a review of the outbreaks in the period from 1992 to 2006 revealed 82 outbreaks related to prepared salads, with 3434 people affected, 66 hospitalisations, and one death. The foodborne illness examples included seven outbreaks of botulism in products of plant origin. The implicated products were commercial garlic-in-oil, hazelnut yoghurt, restaurant potato dip, restaurant aubergine dip, commercial black bean dip, commercial hummus and commercial refrigerated carrot juice. Temperature abuse was suspected to be a contributing factor in four of the outbreaks. In 2007, 55 cases of *Salmonella* Senftenberg infection in England and Wales were linked to fresh basil. Scotland, Denmark, the Netherlands and the USA reported 19 further cases with the outbreak strain. Eight samples of fresh packed basil from Israel tested positive with the same strain. Microbiological evidence suggested an association between contamination of fresh basil and the cases of *Salmonella* Senftenberg infection, leading to withdrawal of basil from all potentially affected batches from the UK market.

Estimating the number of illnesses, hospitalizations, and deaths caused by major pathogens is an important step in the prioritization of pathogens for disease control programs. Estimating the proportions of these illnesses that are due to specific food sources (foodborne illness source attribution) is a necessary second step to determine the specific interventions needed to reduce illness and to measure progress toward public health goals resulting from food safety policies and interventions. Estimates of foodborne illness source attribution are used for many purposes, including informing strategic planning, informing risk-based decision-making, estimating benefits of interventions, and evaluating the impact of interventions.

Plant products as high risk

As previously discussed, the plant products scoping study ranked five specific plant product as high risk due to specific pathogens. These are discussed as follows.

Fresh-cut vegetables and fresh cut fruit

Listeria monocytogenes

Survey data show that *L. monocytogenes* occurs in cut vegetables at low prevalence and usually at low levels. *L. monocytogenes* can grow in a range of vegetables; however growth is typically slow at refrigeration temperatures but numbers can increase by several logs in some commodities stored at 10–15 °C for 7–10 days. The potential for growth in refrigerated short shelf-life products would seem to be low. These products have no final cooking process to eliminate contamination. Where products are packaged by MAP, the potential longer shelf life increases the potential for pathogen growth.

Pathogenic *Escherichia coli*

There is a potential for pathogenic *E. coli* to be present on vegetables via direct or indirect contamination with ruminant faeces or from food handlers that carry the organism in their gut. However, surveys of pre-cut vegetables and salads, other than in Mexico, rarely, if ever, detect pathogenic *E. coli*.

Dose–response for *E. coli*

A very important indicator of the occurrence of an infectious outbreak is the dose response of the pathogen. Gilbert et al. (2006) reviewed the dose response estimates for *E. coli* O157:H7 and the original estimates of the infectious dose were less than a few hundred cells. Later work estimated the probability of infection from exposure to differing numbers of cells. One model predicted a dose of 5.9×10^{5} organisms would result in infection in 50% of consumers, while the probability of illness from 100 organisms was 2.6×10^{-4}. Another study calculated a median dose (50% of people exposed become symptomatic) of 1.9×10^{5} and a probability of 6×10^{-2} of infection when exposed to 100 cells. An analysis of data from the Sakai City elementary school outbreak of *E. coli* O157:H7 indicates much higher probabilities of infection at lower doses than previous models. Gilbert et al. (2006) also reported the dose–response for *E. coli* O111 and O55. The dose for infection of 50% of the exposed population was 2.6×10^{6} organisms. The probability of illness when exposed to 100 cells was 3.5×10^{-4}. Gilbert et al. (2006) state that the organism will grow on leafy vegetables at temperatures above 7°C. However, due to the low infectious dose of the organism in food, growth may not be required to cause illness.

Salmonella

In 2003 the incidence of salmonellae in fruit, vegetables and spices was shown to be below 10%. The numbers of salmonellae on raw vegetables are usually <1 cfu/g, but numbers as high as 240 cfu/g have been found on Dutch endive. Jay et al. (2003) also include information about an outbreak in Germany traced to paprika and paprika powdered potato chips which resulted in an estimated 1000 cases of salmonellosis. The numbers of salmonellae detected in the food were very low, around 2.5 Salmonella cfu/g in the paprika and 0.04–0.45 Salmonella cfu/g of chips.

Clostridium botulinum

The risk of botulism is increased for products packaged using MAP, with the longer shelf life increasing the potential for spore germination and pathogen growth. The contributing factors are the low dose required to cause illness, the severity of the illness, the fact that processing increases the risk and the existence of an epidemiological link. That rating remains appropriate, particularly as longer shelf life vegetable products are becoming more available.

Vegetables in oil

The US Food and Drug Administration (FDA) lists a history of botulism attributed to inadequately acidified foods and notes that products processed by 29 firms were found to be inadequately acidified. The FDA concluded that the evidence demonstrated that certain manufacturers of acidified foods did not realize the importance of adequate pH control. Despite the acidified foods regulation being published in 1979, two serious outbreaks of botulism were reported in the 1980s in Canada and the USA. Chopped garlic in oil was clearly identified as the source of botulism toxin. The concern about vegetables in oil and botulism remains current. The products are popular and home production is common.

According to Food Science Australia (FSA), two false assumptions persist about vegetables in oil:

- *That the addition of oil has a preservative effect*

Incorrect. The only function of the oil is to prevent oxidation from air in the container which can lead to discolouration of some foods. By excluding air from the surface, this establishes anaerobic conditions which actually favor the growth of some types of bacteria, including *C. botulinum*.

- *That some herbs and spices, and especially garlic, have significant anti-microbial properties*

Incorrect. The preservative effect of these materials is slight and inconsistent as outbreaks of botulism in Canada and the USA have demonstrated.

While acidification to a pH less than 4.6 would adequately control the outgrowth of *C. botulinum*, refrigeration is also used in some cases as an additional hurdle.

Seed sprouts

Outbreak investigations have identified several factors that affect the microbiological safety of sprouted seeds. To date, contaminated seeds have been the likely source of most outbreaks.

Seed contamination could have occurred at the farm, seed processor, or sprouting facility. The hydrophobic surface of seeds makes sanitation and removal of contaminating microorganisms difficult. Conditions during sprouting (time, temperature, water activity, pH, and nutrients) are ideal for growth of pathogenic bacteria, leading to an increased risk.

3.2.4. Risk characterisation

Fresh-cut vegetables and fresh cut fruit

➢ ***Listeria monocytogenes***

The FDA/USDA (2003) quantitative risk assessment on *L. monocytogenes* assigned low relative risk rankings to fruits, vegetables, and deli-type salads. While it appears that the probability of infection is low even for persons vulnerable to listeriosis, the consequences of the illness remain severe. The high risk rating is also applied to modified atmosphere products that are stored for extended periods. The potential for growth in storage increases the ranking for MAP vegetables and salads.

➢ ***Pathogenic Escherichia coli***

There have been a number of *E. coli* outbreaks attributed to this group of products around the world. The illness consequences are potentially severe with high rates of hospitalization and long-term effects, such as HUS and kidney problems. Food Science Australia rated the risk as high, while Gilbert et al. (2006) placed pathogenic *E. coli* in the highest severity category but lowest incidence category for New Zealand foods. It was concluded that it is essential that efforts continue to prevent the likelihood of foodborne transmission from this group of organisms.

➢ ***Salmonella serovars***

The risk of *Salmonella* in these high-risk products is based on the severity of the illness and no consumer cooking step to eliminate the hazard. Significant risk of *Salmonella* serovars in fruit and vegetables is based on similar criteria to those defined by FSA, but it should be borne in mind that the increase in their production is not a condition for possible disease. This appears consistent with the many outbreaks attributed to products in which *Salmonella* might survive but not grow.

➢ ***Clostridium botulinum***

FSA rated the risk of *C. botulinum* in these products as high. The contributing factors are the severity of the illness, the fact that processing and packaging using MAP may increase the risk and the existence of an epidemiological link. There is no domestic epidemiological evidence to support the high risk ranking but, to date, longer shelf life vegetable products have had limited availability.

- **Vegetables in oil**

There appears to be clear potential for products prepared without appropriate control measures to result in a poorly acidified product, with potential to cause severe illness from pathogens such as *C. botulinum*. These products are sometimes prepared by small and medium enterprises, which is considered to increase the risk if knowledge of food safety controls is not adequate.

- **Seed sprouts**

The conditions during sprouting (time, temperature, water activity, pH, and nutrients) are ideal for growth of pathogenic bacteria such as *Salmonella* and pathogenic *E. coli*, leading to seed sprouts being considered a high risk product. The potential for growth of pathogenic organisms during sprouting increases the risk substantially, and there is epidemiological evidence to demonstrate that contamination does occur. The implementation of control measures, such as sanitation of seeds prior to sprouting, may lower the prevalence of pathogens.

- **Unpasteurized fruit juice**

The high risk ranking of unpasteurized juice is appropriate. The potential sources of contamination are virtually identical as those in fresh cut fruit, and there is strong epidemiological evidence to justify the risk. The two large-scale outbreaks in Australia, in 1991 and 1999, due to contamination of unpasteurized juice with *Salmonella* serovars have clearly demonstrated the potential for unpasteurized juice to cause illness.

4. Plant food risk management: monitoring, control, and prevention

4.1. Microbiological monitoring

Until the late 1980s, microbiological testing was virtually the only practical hygiene monitoring method available. Using a swab, sponge or contact plate to sample the microbial population on a surface can provide valuable information about the level of contamination.

The sampling device is used to remove microorganisms from a known area (typically 100 cm^2) of a surface such as a worktop or conveyor, or from equipment known to be a potential source of contamination, such as a valve or pump. The material collected is then suspended in a suitable diluent and transferred to a microbiological culture medium, usually in agar plates. After a period of incubation, the degree of contamination can be measured by counting any visible colonies. The main advantage of microbiological monitoring is that it provides a direct indication of microbial contamination, and it is also possible to test for the presence of specific organisms, such as foodborne pathogens.

Unfortunately, there are also a number of limitations, most notably the time taken to obtain results. The culture step normally requires at least 24 hours of incubation and must be carried out in a properly equipped laboratory, which is often off-site. This means that results are only ever available retrospectively and are of limited value for HACCP monitoring purposes, or for rapid cleaning assessment. Even if swabs are taken immediately after cleaning and disinfection, at least a full day of production is likely to have been completed before there is any information available on contamination levels. The results can also be badly affected by residual antimicrobial activity remaining on surfaces after sanitising, so that a special diluent containing inactivating compounds is required to prevent inhibition in the culture.

Nevertheless, microbiological sampling and testing remains a valuable technique, particularly for monitoring vulnerable equipment and fittings for pathogen contamination. Foodborne pathogens like *L. monocytogenes*, *S. enterica*, and *E. coli* are the target organisms in microbiological control according to regulation documents.

4.2. Plant products quality control and prevention

4.2.1. Programs for food safety and quality of plant food

To ensure the food safety of specialty foods, controls should be applied from the beginning to the end of all stages of food production, preparation, and processing to reduce any potential hazards as a means of prevention rather than intervention of finished products.

The following programs are used most often to ensure food safety and the quality of processed foods:

- Good Manufacturing Practices (GMPs);
- Sanitation Standard and Operation Procedures (SSOPs);
- Hazard Analysis and Critical Control Points (HACCP).

➢ **Good Manufacturing Practices (GMPs)**

The GMPs guidelines describe practices for the safe manufacture of foods. They are required by law and apply to all food manufacturing companies. GMPs are prescribed for four main areas of food processing:

- Personnel hygiene to prevent the spread of illness;
- Adequate buildings and facilities;
- Sanitary food-contact surfaces (e.g., equipment and utensils);
- Process controls to prevent cross-contamination.

➢ **Sanitation Standard and Operation Procedures (SSOPs)**

The SSOPs are mandatory for juice and processing plants subject to HACCP. Although specific protocols may vary from facility to facility, SSOPs provide specific step-by-step procedures to help ensure sanitary handling of foods. These documents describe procedures for eight sanitation conditions:

- Safety of water;
- Cleanliness of utensils and equipment;
- Prevention of cross-contamination;
- Hand washing and toilet facilities;
- Protection of food from contaminants;
- Labeling and storage of toxic compounds;
- Monitoring employee health;
- Pest control.

➢ **Hazard Analysis and Critical Control Points (HACCP).**

The HACCP program is a management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement and handling, to manufacturing, distribution and consumption of the finished product. In the 40 years since its inception, HACCP systems have grown to become the universally recognized and accepted method for food safety assurance. The FDA and the USDA have issued regulations that make HACCP mandatory for seafood, juice and meat products as an effective approach to food safety and protecting public health. HACCP is not a stand-alone program. The success of HACCP depends largely on prerequisite programs including GMPs and SSOPs. A foundation of effective prerequisite programs is necessary for HACCP to be successfully implemented.

HACCP involves seven principles:

- Hazard analysis;
- Critical control points (CCP) identification;
- Establishment of critical limits;
- Monitoring procedures;
- Corrective actions;
- Record keeping;
- Verification procedures.

4.3. Food plant sanitation

4.3.1. Sources of contamination

Assuming the bacteria are not internalized in the plant tissue, surface sanitisation is normally an effective way to remove pathogenic bacteria from products. Many reports show that contaminated surfaces are most likely the source of product contamination, and that the risk of contamination is highest between the primary trimming and chopping and the packaging stages. Sources of contamination that should be considered are: hands, gloves, and personal protective equipment; slicers; conveyors; washing tanks and wash water; holding containers and racks for packaging, and packaging equipment.

Cross-contamination is one of the main risks to food safety during processing. Cross-contamination can occur in three main ways:

- **Food to food.** Food can become contaminated by bacteria from other foods. This type of cross-contamination is especially dangerous if raw foods come into contact with cooked foods.
- **People to food.** People can be a source of cross-contamination to foods, when good hygienic practices are not performed by food handlers, such as handling foods after using the toilet without first properly washing hands, touching raw meats and then preparing foods without washing hands between tasks, using an apron to wipe hands between handling different foods, or wiping a counter with a towel and then using it to dry hands.
- **Equipment to food.** Contamination can be passed from kitchen equipment and utensils to food. This type of contamination occurs because the equipment or utensils are not properly cleaned and sanitized between each use.

Temperature during storage and distribution can also be a source of contamination. Disease-causing pathogens can grow well in food when it is kept at temperature between 5°C and 57°C, which is known as the temperature danger zone. If food is kept at these temperatures for more than 4 hours, pathogens can grow to levels high enough to cause serious food-borne illnesses. Therefore, it is very important to keep hot food at 60°C or higher and cold food at 5°C or lower, and check the food's temperature at least every 4 hours during storage and distribution.

To prevent or reduce the amount of pathogens, different methods of sanitation are implemented. Sanitation is a multistep process that involves cleaning and sanitizing as two very important and separate steps. Effective cleaning and sanitation procedures, which include debris removal, use of detergent solutions, rinsing with water, disinfection where necessary and dry cleaning, are required to achieve the correct level of hygiene in food-handling or production facilities. If these are not adhered to, there is a greater risk of food becoming contaminated by pathogenic or spoilage microorganisms. There is also a risk of biofilms forming on factory and food preparation surfaces if these programs are inadequate. It is important to avoid the use of phenolic and metal-ion based products as they may cause product tainting and consumer safety issues.

Cleaning and sanitation programs include four steps:

- Routine procedures performed throughout and at the completion of food processing or preparation on a daily basis;
- Periodic procedures required less frequently;
- Monitoring to ensure the procedures are performed correctly;
- Verification to check the effectiveness of the program.

4.3.2. Methods for sanitation

Disinfection

Many producers and suppliers are committed to good practices to reduce the risk of contamination. Conventional methods to decrease contamination include post-harvest decontamination procedures. However, experiments using standard post-harvest decontamination procedures with solutions containing approximately 20–200 mg ml^{-1} free chlorine for various lengths of time found that bacterial numbers were reduced but the treatments did not completely eliminate either the natural microbial population, or human pathogens.

Household natural sanitizers including fresh lemon juice and vinegar have been shown to have some effect in the reduction of *Salmonella* serovar Typhimurium on rocket leaves and spring onion. A 15 min treatment with 1:1 lemon juice and vinegar reduced viable counts to undetectable levels. Treatment of carrots with this solution reduced *Salmonella* CFUs to an undetectable level.

Treatment of commercial iceberg lettuce pre-inoculated with natural spoilage organisms with chlorine, ozone or a combination of them reduces the number of viable microorganisms. Additionally chlorine–ozone combinations increase the shelf life of lettuce. No visible changes in rinse water turbidity or reduction in quality are observed during rinsing of the lettuce, indicating applicability to commercial processing.

Ionizing radiation has demonstrated efficacy in reducing microbial contamination. In particular, studies focused on leafy greens have shown multiple log reductions in *L.*

monocytogenes, *Salmonella* and *E. coli* O157:H7 when used on various leafy greens, including iceberg lettuce, Romaine lettuce, and spinach.

Biocontrol

An alternative method to reduce contamination would be the use of agricultural practices that encourage growth of competing bacteria within the phyllosphere to reduce the contamination with human pathogens. Various studies suggest that the natural microflora of plants can inhibit the growth of *E. coli* O157:H7, *Salmonella* serovars Montevideo and Chester, and *Staphylococcus aureus*. *Pseudomonas* and *Bacilus* species isolated from green pepper, Romaine lettuce, baby carrots, alfafa, and clover sprouts can inhibit the growth of *Salmonella* serovar Chester and *L. monocytogenes*. *Enterobacter cloacae* reduced the colonization of carrots, cress, lettuce, radish, spinach, and tomatoes by *E. coli* O157:H7 and *L. monocytogenes*, whereas *Enterobacter asburiae* decreased their survival on lettuce. Also, growth of *Arabidopsis thaliana* with *E. asburiae* in gnotobiotic conditions strongly reduced the root contamination by *Salmonella* or *E. coli* O157.

Image-based control

Identification and quantification of plant diseases are required for adequate plant protection, determination of crop losses, and for the design of breeding strategies in agriculture. The potential of image-based analysis in the detection and monitoring of plant disease is well accepted and the use of these techniques to study plant infections with potential human pathogenic microorganisms is a new and developing area. This analysis involves capturing targets arrays of plant production and search of phenotypic marks in plants, which are an indication of phytopathogenic diseases or the development of human pathogens.

4.3. Examples of implementing specific food safety controls in specialty foods processing

Specialty Fruit Juice

Specialty fruit juices, such as pomegranate juice, mangosteen juice, and noni juice, are often claimed to contain a high content of health-promoting phytochemicals, including antioxidants and bioflavonoids. When a health claim is made on the label of juice products, the labeling should follow the FDA's food-labeling regulation on health claims. If improperly handled, fruit juices can harbor pathogenic microorganisms and have been associated with food-borne illness outbreaks. The FDA has issued regulations that mandate the application of HACCP principles to the processing of fruit and vegetable juices. The HACCP plans shall include control measures that will consistently produce a minimum of 5 log reduction, for a period at least as long as the shelf life of the product when stored under normal and moderate abuse conditions, in the pertinent microorganism. For the purposes of this regulation, the "pertinent microorganism" is the most resistant microorganism of public health significance that is likely to occur in the juice, e.g. *Escherichia coli* O157:H7.

Pasteurization is a critical control point in juice processing. The heat process used in pasteurization increases the shelf life of juice by inactivating microorganisms and certain enzymes. To better maintain the color and flavor, flash pasteurization, also called *high temperature short time* (HTST) processing, is widely used for fruit juices. It will provide a safe product for the public, yet keep to a minimum the amount of flavor-degradation found in ultra-pasteurized products. In flash pasteurization, the minimum temperature used is 71.5 °C for a holding time between 15 and 30 seconds. To ensure the success of pasteurization, the temperature of juice needs to be continuously monitored by a temperature recorder during the pasteurization process. When the monitoring indicates a deviation from the established critical limit, juice producers must segregate and hold the affected product for evaluation, destroy it, or divert it to non-food use, and adjust the pasteurizer (temperature or flow rate) to achieve the critical limit. The accuracy of the temperature-recording device needs to be checked daily against a mercury and glass thermometer. The mercury and glass thermometer should be annually calibrated.

Control measures for ensuring the safety of specialty fruit juices

- A supplier guarantee must exist to specify that the shipment includes only fruit harvested to exclude fallen fruit.
- The fruit must be rinsed and then brush washed with a sanitizer containing minimum 200 ppm of available chlorine for a contact time of 30 seconds.
- A pasteurization process with a minimum temperature of 160 °F (71.5 °C) for 15–30 seconds is required to provide a 5-log reduction of the pertinent pathogen. The heating temperature and time are critical factors that must be monitored, controlled, and documented.

5. Food safety awareness, messages, and knowledge

What is the state of awareness of modern society in terms of food safety? A survey of students and working people showed different levels of awareness. On investigating food safety awareness, the majority of respondents (76%) indicated that it was "very safe" to wash hands after touching raw plant/chicken/meat/fish. Also 50% of the students indicated that it was "very safe" to use separate cutting boards or knives for raw food products. These showed a significantly high level of food safety awareness among the students.

The female students at the secondary level showed a significant higher level in terms of lack of awareness for cross-contamination and positive food safety awareness habits compared to the male students. The female students at the tertiary level showed a significant lower level of awareness for cross-contamination than their male counterparts. Overall, the majority of students showed a lack of awareness of cross-contamination. The majority of males at the secondary level were unaware of the risks associated with the area of cross-contamination.

The mean awareness score was (16/50) at the secondary level and at the tertiary level, it was 15/50. The range of awareness scores for secondary students was 0–35 out of 50 and 0–30 out of 50 for the tertiary level students. The male students at the tertiary level showed a higher level of practicing food safety awareness and scored awareness. Also, the food safety awareness median (15/50) and mode (20/50) scores were the same at the secondary and tertiary level. At the secondary school level, 50% of females and 6% of males responded "yes" to seeing, hearing, and reading information about food safety apart from this questionnaire. These students participated in Home Economics and Food and Nutrition classes. At the tertiary level, 21% of males and 17% of females also indicated "yes". Of the n = 205 respondents, 15% of the females and 11% of the males indicated "no" to seeing, hearing, and reading information about food safety apart from this questionnaire and 13% of females and 10% of males indicated that they cannot recall seeing, reading, or hearing anything about food safety. Eighteen percent (18%) of respondents at the tertiary level stated that they got food safety information from the television and the Internet.

Further analysis as to where students heard or read information about food safety revealed that 36% of females and 14% of males got information from various other sources such as newspapers, books, parents, and food labels. At the secondary school level, 52% of the female students and 5% of the male students indicated that they had formal training in food safety or food preparation. At the tertiary level, 56% of the males and 36% of females indicated that they had formal training in food safety or food preparation.

Participants were not scored on the knowledge questions, since these questions were structured to determine the participants' self-assessment of their food safety knowledge**.** The self-assessment of perceived food safety knowledge of 23% of females and 3% of male respondents at the secondary and 7% of female and 12% of male respondents at the tertiary level revealed that they felt that their knowledge base of food safety was "very good". A further breakdown by gender demonstrated that 43% of the females and 1% of males at the secondary level, together with 25% of females and 27% of males at the tertiary level, felt that their food safety knowledge was "quite good". The males at the tertiary level showed a higher perceived knowledge level than their female counterparts and the males at the secondary level. The male students at the secondary level showed a significant lower knowledge level than their female counterparts. There was no significance between the females at both institutions. Overall, at the secondary level, the female population and the male population at the tertiary level showed that the knowledge base of these students reflected knowledge of food safety issues.

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